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13. ABSTRACT *(Maximum 200 words)*

Specific Aims: to test two hypotheses of using electrochemical treatment (EChT) for cancer therapy: 1) both anodes and cathodes should be inserted inside the tumor; 2) tumor responses are dependent on electrode spacing and dose. **Methods:** Rat breast cancers were initiated by injecting 1 x 10^6 MTF-7 cells to the rat right mammary gland fat pad. Rats were randomly divided into experimental groups when the tumors were grown to 2 x 2 x 2 cm^3. 69 rats were used for hypothesis 1 study, 130 for hypothesis 2 - survival study and 129 for hypothesis 2 - pathological study. **Results:** The tissues around electrodes were necrosis after EChT. The effects of EChT are non-specific since it destroys both tumor and normal tissues. The survival study indicated that the tumor control and rat survival rates significantly increased with increasing dose regardless of the spacing. The local control rate is less than 40% in 40 C and 60 C groups and more than 70% in 80 C and 100 C groups. 66 rats died of primary tumor including 10 rats in control group. Once the primary tumor is controlled, no recurrence was found in this study. The main reason to terminate the primary tumor-free rats (51 rats) was lymph node metastasis. 13 rats survived over six months tumor free. Pathological study showed that there was a significant dose effect on the EChT induced tumor necrosis. At 10 C, 20 C, 40 C, and 80 C, the percent of the necrosis were 39.7%, 52.3%, 62%, and 77.7%, respectively ($p < 0.00001$). Spacing is not an important factor within a given range. At 5, 10, and 15 mm spacing, the percent of the necrosis were 54.1%, 60.4%, and 59.2%, respectively ($p = 0.552$). The necrosis overlap rate was similar at 5 mm and 10 mm groups (82.5% and 85%), while lower in 15 mm group (65%). **Conclusions:** The effects of EChT are non-specific, the cathodes should not be inserted into the normal tissues. For a diameter of 2.0 to 2.5 cm rat breast cancer, EChT with 5 - 10 mm spacing and at least 80 C are the best parameter combinations.

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INTRODUCTION

The grant "Electrochemical Treatment of Breast Cancer with Direct Current" is a two-year project, from February 1, 1997 to March 1, 1999. A no cost extension was approved to complete the grant on June 1, 1999. Dr. C.K. Chou was the initial Principle Investigator. In April of 1998, Dr. Chou left the City of Hope National Medical Center and Dr. Nayana Vora became the grant Principle investigator.

The electrochemical treatment of cancer (EChT) has been suggested as an effective alternative local therapy (1 - 7). However, the methodology is not fully-established and mechanisms are not well-studied. To verify its effectiveness and further make EChT a useful alternative method for treating localized cancer, the City of Hope National Medical Center started EChT study in September of 1993 with a seed grant from the National Institute of Health, Office of Alternative Medicine. *In vivo* studies were conducted to evaluate the effectiveness of EChT on animal tumor models. Treating mouse and rat fibrosarcomas with proper levels of direct current, we found that inserting both anodes and cathodes into tumors especially at the base of the tumor could result in tumor-free long-term animal survival (7). *In vitro* studies on human KB cells also showed that EChT inhibited cell proliferation and DNA synthesis (8). Morphological results were consistent with the cell culture observations (9). Those results encouraged us to conduct more rigorous research to provide a solid foundation for this promising, simple, and economical alternative therapy for treating localized tumors, such as the breast cancer.

The specific aims of this grant are to test two hypotheses: 1) both anodes and cathodes should be inserted inside the tumor; placing electrodes in normal tissue will cause severe damage, 2) tumor responses are dependent on electrode spacing and dose. The goals of the study are to provide fundamental knowledge about EChT electrode configuration, spacing, and dose and to help formulate a standardized EChT method for treating breast cancer.

MATERIALS AND METHODS

Rat breast cancer cells:

There are three kinds of rat breast cancer cells in our laboratory, MTLn3, MTC, and MTF-7. Those breast cancer cells were obtained from the M.D. Anderson Hospital and Tumor Institute. Initially, we proposed to use MTLn3 to create a tumor model in Fisher 344 rats, as we described in the proposal. However, our preliminary study indicated that when MTLn3 tumor grew to 2 x 2 x 2 cm^3, obvious necrosis appeared. This necrosis introduced complicating factors into the study and made it difficult to determine the effectiveness of EChT. The same problem happened in MTC rat breast cancer model. Only MTF-7 breast cancer can grow to 2.5 x 2.5 x 2.5 cm^3 without obvious necrosis. Therefore, we used MTF-7 cell line instead of MTLn3 cell line in our studies.

The MTF-7 cells were grown *in vitro* in MEM Eagle with Earle's Salts and L-glutamine (200 mM). The solution was supplemented with 10% heat-inactived fetal bovine serum and 1% penicillin/streptomycin (10,000 units/ml penicillin G and 10,000 μg/ml streptomycin sulfate in normal saline). When the cells reached approximately 80% confluence, they were collected and suspended in PBS for injection. The final concentration for the injection was 1 x 10^6 cells/0.25 ml.

Tumor model creation:

Fisher 344 female rats, with a weight range 45 – 160 grams, were used in this study (provided by Charles River). An animal protocol for the study was approved by the City of Hope Research Animal Care Committee. Rats were housed *ad lib* at the City of Hope Animal Resource Center accredited by the American Association for the Accreditation of Laboratory Animal Care. Housing and care of animals are consistent with the Public Health Service policy, the Guide for

the Care and Use of Laboratory Animals, the Animal Welfare Act and other applicable state and local regulations. 0.25 ml of the MTF-7 cell suspension (1 x 10^6 cells) was injected subcutaneously beneath a nipple in the right chest of the rat. When the tumor reached to approximately 20 mm in diameter, the animal was ready for EChT.

Animal Groups:

Hypothesis 1: A total of 69 rats were used for hypothesis 1 study. Sixty rats were used to determine the effects of different doses used on tumor tissue. The remaining nine rats were used to examine the effects of EChT on normal muscle tissue. For tumor responses to EChT, 60 rats were divided into the following groups: control, 5 coulombs (C), 10 C, 20 C, 30 C, and 40 C. The 5 C and 20 C groups had 12 rats in which 9 were for light microscope (LM) studies and 3 were for transmission electron microscope (TEM) studies, other groups had 9 rats for each and only underwent LM studies. The control groups were shared for both LM and TEM studies. For normal muscle tissue response to EChT, 9 rats were divided into 3 groups: anode insertion, cathode insertion, and both anode and cathode insertion. Three rats were used for each group.

Hypothesis 2 – survival study: One hundred twenty rats were randomly divided into three groups and the electrode spacing was 3 mm, 5 mm, and 10 mm, respectively. In each group, the 40 rats were divided into 4 subgroups of 10, each group treated with one of four levels of 8-V constant voltage EChT: 40, 60, 80, and 100 C, respectively. Additional 10 rats served as controls. These control rats were anesthetized and electrodes with 5 mm spacing were inserted into each tumor like the other rats, but no voltage was applied.

Hypothesis 2 – pathological study: One hundred twenty nine rats were randomly divided into three groups and the electrode spacing was 5 mm, 10 mm, and 15 mm, respectively. In each group, the 43 rats were divided to 4 experimental subgroups of 10 and 1 control subgroup of 3. Each experimental subgroup treated with one of four levels of 8-V constant voltage EChT: 10, 20, 40, and 80 C, respectively.

ECht Instrument:

A four-channel instrument (BK92A: Beijing University of Aeronautics and Astronautics, Beijing, China) was used for this study. This is a computer-based direct current power supply with maximum voltage (V), current (mA), electrical charge (C), and time (min) settings. The treatment may be set to a constant-voltage or a constant-current mode. Since clinical studies indicate that treatment at high voltages can cause pain to the patients, the constant-voltage setting was used. The instrument has the capability of slowly "ramping" up to the set voltage (or current, if the constant-current model is used) to prevent shocking the patient. Open-circuit and short-circuit resistance warnings and the rate of voltage increase may be set individually. A maximum of 4 rats can be treated at the same time.

Electrodes:

The 24 gauge (~ 0.53 mm diameter), pure platinum wire (Model 9924-F50) was purchased from Thomas Scientific, Swedesboro, New Jersey. The wire was cut into 6-cm fragments and the tips were sharpened to facilitate insertion. Catheters were used for initial tumor penetration since the platinum electrodes were too soft for rat skin. The electrodes were cleaned and sterilized in 70% EtOH prior to use.

ECht procedure:

Before ECht, rats were anesthetized intraperitoneally with a 3:1 Ketamine:Xylazine cocktail. Plastic plates with slots were used to lift the tumor away from the body. The eyes of the rats were protected from dehydration with an ophthalmic antibacterial ointment. Once the rats were anesthetized, for hypothesis 1 study, two platinum electrodes were inserted vertically (perpendicular to the plane of the body) into the tumors at a 10-mm spacing and connected to the anode of the ECht instrument, respectively. Additional parameters were configured as follows: 8 V, a maximum of 80 mA, and a maximum treatment time of 120 min. For normal muscle tissue

10 C dose was applied. While anode or cathode was inserted in the muscle, the other electrode was inserted in the tumor. While both anode and cathode were inserted in the muscle, the space between two electrodes was 10 mm.

For hypothesis 2 - survival study, electrodes were inserted parallel at the base of the tumor. The insertion was perpendicular to the long axis of the body. An arrangement of alternating cathodes and anodes was utilized. The numbers of the electrodes were dependent upon the tumor size and the designed spacing, 3 mm, 5 mm, or 10 mm. A constant voltage of 8 V was applied at 0, 40, 60, 80, and 100 C, respectively. For hypothesis 2 - pathological study, two electrodes were inserted vertically to the tumors with pre-designed spacing, 5 mm, 10 mm, and 15 mm. A constant voltage of 8 V was applied at 0, 10, 20, 40, and 80 C, respectively.

Follow-up and data collection:

Sample collection for hypothesis 1 study: Upon completion of the treatment, tumor samples were removed immediately, 24 hours, and 48 hours following treatment for LM studies. For TEM and muscle response studies, the samples were removed immediately following EChT. Three samples were collected at each time point from each group and each rat provided only one sample. Light microscopy samples were prepared with standard H&E staining procedure. Transmission electron microscopy samples were prepared following a 4% paraformaldehyde/1.7% glutaraldehyde procedure.

Hypothesis 2 - survival study: After EChT, rats were returned to the animal housing facility. To prevent the rats from cannibalizing one another's tumors, rats were housed separately during recovery; then two rats, after the tumor regressed, were housed in one cage. The tumor wound was cleaned daily with hydrogen perioxide and Betadine ointment until the scab fell off. Tumor volume was originally intended as an evaluation parameter. However, the shapes of treated tumors became irregular and it was impossible to obtain accurate measurements of tumor sizes. Therefore, tumor local control and survival were used as the ultimate determinant. In

addition, rat health was monitored daily to check for tumor recurrence and metastasis. As indicators of health, body weight and lymph node were monitored daily for the first three months, then twice weekly for the next three months.

Hypothesis 2 - pathological study: Tumors were dissected after EChT. The specimen (tumor mass) was fixed in Formalin (10% neutral buffered formaldehyde). They were individually examined and oriented according to the electrode plane (positive in red and negative in black). The specimen was then weighed and measured in three dimensions. Serial 2-mm sections were made through the specimen parallel to the electrode plane so that each section contains both positive and negative electrode fields. One center section at the prime plane was selected for tissue processing. The selected tissue was processed on an automated tissue processor following the instructions provided by the manufacture. Glass slide was made from 2 – 3 □m-thin sections on paraffin embedded tissue after tissue process as routinely performed in any conventional histochemistry laboratory. The slides were then stained with hematoxylin-eosin (H&E). H & E stained slides were examined under an Olympus light microscope. The maximum dimension of necrosis was measured microscopically. The percent of necrotic area on the entire section was estimated. Special attention was placed on the relationship of the necrotic areas surrounding each electrode and a notion was made whether the two coalesce. The initial examination was performed in a blind fashion.

Calculation of current density:

Current densities around the electrodes were calculated using the impedance method (10, 11). The calculation was based on a 5 cm square box filed with 1 cm depth 0.9% saline solution. The conductivity 1 S/m was used for the calculation. Two 0.525 OD electrodes at 1 cm separation were inserted into the solution at 5 mm depth.

Statistical Methods:

Hypothesis 1 study compared the morphological changes of EChT on tumor and normal tissue. No quantitative data was generated. Data analysis was performed for hypothesis 2 of technical objectives.

Separate sets of animals were used for pathology and tumor local control/survival studies. Both used factorial combinations of spacing and dose, and the latter used a zero dose, which was treated as a separate treatment category (spacing being irrelevant). The effects of dose and spacing on percent necrosis were evaluated using the Kruskal-Wallis rank sum test and by multiple linear regression. The effects of dose and spacing on the probability of overlap of necrotic regions, and on the probability of no necrosis, were evaluated by Pearson chi-square tests and logistic regression. For each endpoint, an omnibus test was used to control the experiment-wise false positive rate, followed by more focused trend tests. Tumor control was analyzed by logistic regression, and proportional-hazard (Cox) regression models were used to analyze survival.

RESULTS

Hypothesis 1: Detail results and pictures of the hypothesis 1 study have been presented in the annual report submitted in March of 1998. The results are summarized below.

Naked eye observation: during EChT, the color turns to black in the anode area and reddish in the cathode on skin of tumor site while yellow in the anode and white in the cathode inside of tumor. Bubbles were observed around the cathode. The tumor tissue shrank and presented caseation in anode area and congested and swelled in cathode area. Twenty-four hours after EChT, the entire tumor shrank and a scar was formed at the tumor site. The wound gradually recovered by the formation of skin over the treatment area in a few days after EChT.

Light microscope (LM): EChT could result in distinct changes to the cellular structure of

the treated tumors. In the anode area, the nuclei shrank and the cytoplasmic structure disappeared. In the cathode area, the cell swelled and the interface among cells blurred. The extent of cell structure disruption was related both to the condition of the tumor at the time of treatment and the dose applied during treatment. These changes ranged from blurred to complete loss of cellular outlines. An abrupt transition from treated tumor to viable tumor indicated that there is a definite effective treatment area, based upon the dose applied. The area adjacent to the cathode exhibited minor swelling, while dehydration was observed around the anode. Also, we observed disruption in the treated muscle tissue, either with cathode, anode, or both.

Transmission electron microscope (TEM): Even though at a low level of 5 C EChT, TEM showed that cells shrank, mitochondria swelled mildly and the crest disappeared, polysome disaggregated, lysosome distended, and nuclear chromating aggregated focally in anode area while cells swelled and nuclei vacuolized in cathode area. At a higher level 20 C, plasma membrane burst and the distended organelles escaped in both anode and cathode areas. Microscopic studies reveal morphological changes corresponding to inhibition of cell proliferation after EChT.

Hypothesis 2 - survival study: Before EChT, the tumor volumes were well-balanced across both experimental factors: spacing and dose (Table 1). During follow-up, rats' weight did not show significant change in all groups (Table 2). All rats were successfully treated with EChT, except one died at the second day after EChT. Autopsy showed that the rat death was stress related. The tumor control rates of 12 experimental groups are shown in the Figure 1. With increasing dose, the tumor control rates increased. In 80 C and 100 C groups, the tumor control rates were more than 70%, while less than 40% in 40 C and 60 C groups regardless of the spacing. Total local control rate was 53.3% (64/120) in experiment groups. None achieved local control in the control group. There is no difference in tumor dropping off time. In summary, there is a clear increase in tumor control rate with increasing dose ($p < 0.0001$). There was no significant change in tumor control rates associated with the electrode spacing ($p = 0.16$).

Once the primary tumor came off, no recurrence was found in this study. However, most of the primary tumor-free rats developed lymph node metastasis. It was the main reason to euthanatize the rats. Sixty-six rats died of primary tumor including 10 rats in the control group. Fifty-one rats died of lymph node metastasis. Thirteen rats survived over six months wwre tumor-free. Table 3 shows the survival results. Fitting Cox proportional hazard models indicated that there was a significant trend (linear $p << 0.000001$) in dose, with significant curvature (quadratic $p = 1.8e^{-0.06}$), but no significant effect of spacing.

Hypothesis 2 - pathological study: One hundred twenty nine slides from 129 tumor masses were examined. After the evaluation in a blind fashion, all slides were re-examined to assess the pattern of necrosis. In the control group (0 dosage), varying percentage of scattered necrosis was noted with highly irregular size and shape. They were admixed with areas showing ischemical changes and islands of regenerating tumor cells. There was a lack of well-defined necrotic zone surrounding the electrode. No liquefaction was present. As described in the hypothesis 1, the EChT induced necrosis characteristically showed zonal necrosis surrounding the electrode. The necrosis surrounding the positive electrode was coagulative in nature with preservation of the architecture contours. In contrast, the necrotic area surrounding the negative electrode exhibited liquefaction and complete effacement of the tissue architecture.

One problem we encountered in the analysis was the varying degree of non-EChT induced necrosis in the EChT treated specimen. They were highly irregular in size, shape and extent (percentage of involvement). They often coalesce with EChT induced necrosis. This appeared to be less problematic in the high dose groups when EChT induced necrosis predominate. The maximal dimension of the necrosis microscopically was originally intended as an evaluation parameter. However, due to the necrosis being highly irregular in size and shape, we analyzed the percentage of necrotic area on the entire section and the necrosis overlap rate across both dose and spacing factors as ultimately determinant (Tables 4 and 5). Kruskal-Wallis rank sum test and multiple linear regression were used to evaluate the effects of dose and spacing on percent necrosis. For dose factor, Kruskal-Wallis chi-square was 44.3 and p-value was < 0.00001. For

spacing factor, Kruskal-Wallis chi-square was 1.19 and p-value was 0.552. There was a highly significant dose effect on the EChT induced tumor necrosis. Percent necrosis increases by about 0.5 percent per Coulomb (within the range used). Pearson chi-square tests and logistic regression were used for analyzing the effects of dose and spacing on the probability of overlap of necrotic regions. Since chi-square in not powerful for detecting a trend, Logistic regression is preferable, assuming a more-or-less linear trend is a reasonable a-prior expectation for dose-response. Table 5 showed that there was weak evidence (non-significant by conventional levels) of a decrease in the rate of overlapping necrosis regions at the widest spacing and reasonably solid evidence of a trend toward increasing probability of overlapping necrosis regions with increasing dose.

Calculation of current density: Figure 2 shows the current density pattern at layer 10 which is at the center of the immersed electrode. The peak value was about 35 mA/cm^2 at the surface of the electrodes. The current density drops very sharply to small values (less than 2.5 mA/cm^2) within 2 mm. The current density patterns remain about the same except at the very tip of the electrodes.

DISCUSSION

Since Nordenström, a Swedish radiologist, treated 26 lung metastases in 20 patients with direct current and developed his "biologically closed electric circuits (BCEC)" theory (1, 12 - 14), direct current therapy for cancer which was named as EChT later induced much interest in the scientific and medical community (2, 4, 5, 7, 12, 15). Particularly in China, EChT was used in 1260 hospitals throughout the country and more than ten thousand patients with various cancers were treated (2). Dr. Xin summarized the results of 7642 malignant tumors which included skin cancer (958), lung cancer (1113), liver cancer (961), and breast breast cancer (644). The 5-year survival rates were 80%, 38.8%, 15.1%, and 50.2%, respectively (2). The local control rates were considered satisfactory in comparison with conventional therapy, but produce minimum trauma as compared to the convention therapy. Although EChT is already clinically prevalent in China and a number of

clinical studies have confirmed that EChT has an antitumor effect, EChT is not a well-established method. Review of pertaining literature shows that precise guidelines regarding electrode insertion and electrical parameters (i.e. electrode spacing and dosage) are not available.

At this time, published clinical studies show various electrode insertion methods and distributions; different electrode placements have been used in Europe and China. Nordenström (1, 14) developed BCEC theory and viewed the tumor is treated with EChT by two processes: 1) EChT activates ionic transports and reactions between electrodes in tissue, and 2) injuries produced, which also activate the nutritional BCEC channels of the cancer, thus initiating the healing process. The flow of ionic current triggers interactions between the induced electromagnetic fields and the cancer tissue. Therefore, Nordenström has been treating his lung cancer patients with the anode inserted in the tumor center and cathode in normal tissue several centimeters away from the tumor boundary. European researchers and physicians have followed this method (16, 17).

In China Dr. Xin modified the technique, putting both anode and cathode into the tumor, with the anode in the center and cathodes in the periphery (18). Chinese physicians have found that by placing both the anodes and cathodes in tumors not only protected the normal tissue but also had a greater effect on the tumor. However, there are no research reports on the degree of normal tissue damage and the differences between the damages near anode and cathode. Whether both the anode and cathode should be inserted within the tumor or the cathode should be in normal tissue, a detailed animal study should be conducted to test whether induced cell damage occurs around the anode and cathode during EChT. Previous studies indicated that near the anode, the tissue is acidic and dehydrated. Near the cathode, the tissue is basic and hydrated (8, 19). Our results in this study obviously indicated that both anodes and cathodes could destroy the tumor cells and induce necrosis of tumor tissues. Furthermore, both anodes and cathodes can also induce degeneration and necrosis of muscles. Based on these observations, we infer that both the electrodes will destroy treated tissues. Therefore, inserting cathodes into normal tissues should be deterred because it will result in unnecessary damage of normal tissues. This complication may be totally avoided by inserting both anodes and cathodes into the tumor tissues.

Another important issue to be studied is to decide the optional electrode spacing and dosage. We used the impedance method to calculate the current density during EChT since EChT involves electrolysis, electrophoresis and electroosmosis (19 - 22), and the effective volume of the treatment is limited to the vicinity of the electrodes. The high current density around the electrode was limited about 2 mm. Beyond this region, the density drops sharply. Therefore, it was thought that to cover the tumor region with an adequate number of electrodes is essential. If the spacing is more than the effective region, it will cause the tumor recurrence after EChT. If the spacing is too small, the procedure is difficult and it will induce open-circuit and short-circuit during EChT. A great variety of electrode spacing has been employed to date. In China it was reported that the effective volume around each electrode in lung cancer is about 2 - 3 cm in diameter; therefore, they suggested that the spacing between electrodes be kept at less than 2 cm for lung cancer (18). For benign tumor and superficial tumors, 1 - 2 cm spacing was used (23, 24). Japanese scientists have used 1 to 2 cm spacing for animal studies and 3-4 cm spacing for clinical study, but no reason was given for the choices (4). While in Slovenia, the cathode was inserted in the skin tumor, and a plate electrode was pasted on the skin at 3-4 cm from the edge of the melanoma skin lesion (25). All those recommendations are based on their own experience and lack detail research. Since each tumor has its own tissue conductivity, the effective volume differs for each different tumor. For rat breast cancer, our preliminary studies have shown that if the spacing is more than 1.0 cm, tumor recurrence was observed. To determine the optional spacing for rat breast cancer EChT, we set three different spacing levels, 3, 5, and 10 mm to compare the tumor control and rat survival rates. We found that the spacing 3, 5, and 10 mm did not significantly affect the control and survival rates if given the same dose. In addition, we found that it was too difficult to perform with 3 mm spacing during electrodes insertion. Short-circuit and open-circuit happened during EChT and the treatment was interrupted. Therefore, we concluded that both 5 mm and 10 mm spacing could achieve satisfactory results and easy to perform for rat breast cancer.

The EChT dosage guideline is arbitrary to date. Electric dose (coulombs) is a product of current (A) and time (sec). Higher current reduces treatment time but also causes pain. Lower

current prolongs the treatment. Therefore, a compromise with acceptable current density over a reasonable time period is desirable. 100 C per cm of tumor diameter (at 10 V potential) was used in Nordenströom's studies (1, 13 - 14). Matsushima et al. (4) treated tumors of similar sizes (about 6 cm x 6 cm) with 40 to 960 C. Xin (2, 18) treated his patients with 40-80 mA current, 8-10 V, at 100 C per cubic centimeter. The 100 C per cubic centimeter is more appropriate than per centimeter diameter, since the dose should be related to the volume and not the diameter of the tumor. Although 100 C has been widely used, there is no scientific basis for these numbers.

In this study, the tumor diameter was approximately 2.0 to 2.5 cm. The tumor volume before EChT was well balanced in all groups, approximately 5.2 ± 1 cm^3. For survival study, we set four level dose groups in three spacing: 40 C, 60 C, 80 C, and 100 C. The result indicated that there was a clear increase in the tumor control and rat survival rates with increasing dose regardless of the spacing. There was no significant decrease in tumor response to EChT associated with electrode spacing change. The local control rate is less than 40% in 40 C and 60 C groups and more than 70% in 80 C and 100 C groups. The groups 80 C and 100 C achieved the best results. This dose is much less than either Dr. Xin's or Dr. Nordenströom's suggestion. However, it should be pointed out that the extrapolation of EChT dose guide from animals to humans is not completely reliable.

The results of the pathological study were consistent with the survival study. As dose increased, the percent of the tumor necrosis and the necrosis overlap rate increased. There was a highly significant dose effect on the EChT induced tumor necrosis. At 10 C, 20 C, 40 C, and 80 C, the percent of the necrosis were 39.7%, 52.3%, 62%, and 77.7%, respectively ($p < 0.00001$). The necrosis overlapping rates at low dose groups (10 C, 20 C, and 40 C) were lower than at high dose group (80 C). At 80 C group, there was 93.3% treated rats showed necrosis overlapping. The spacing is not an important factor within given range. At 5 mm, 10 mm, and 15 mm spacing, the percent of the necrosis were 54.1%, 60.4%, and 59.2%, respectively ($p = 0.552$). The necrosis overlap rate was similar at 5 mm and 10 mm groups (82.5% and 85%). However, there was weak evidence (non-significant by conventional levels) of a decrease in the rate of

overlapping necrosis regions at the widest spacing (65% at 15 mm spacing). Combing the survival and pathological studies, EChT with 80 C to 100 C and 5 mm to 10 mm spacing is a suitable treatment for rat breast cancer.

Table 1. Tumor volume before ECT

Tumor volume by dose						
Dose (C)	0	40	60	80	100	
Rat number	10	30	30	30	30	
Volume						
Mean	5.04	5.20	5.26	5.16	5.06	
Std Dev.	0.97	1.02	1.11	0.99	1.36	$P > 0.01$

Tumor volume by Spacing -- excluding dose: 0 (control)				
Spacing (mm)	3	5	10	
Rat number	40	40	40	
Volume				
Mean	5.21	5.22	5.08	
Std Dev.	1.11	1.15	1.12	$p > 0.01$

Table 2. Rats weight change during follow-up

Time (day)	0	10	20	30
Group (mm/C)				
3/40	162.4 ± 1.8	165.6 ± 0.9	164.3 ± 1.4	163.2 ± 1.8
3/60	159.8 ± 0.9	159.4 ± 1.1	162.5 ± 1.0	161.4 ± 1.1
3/80	161.5 ± 1.2	161.7 ± 1.0	161.9 ± 0.9	162.4 ± 1.0
3/100	160.5 ± 1.1	159.8 ± 1.1	161.4 ± 0.9	162.5 ± 1.1
5/40	163.6 ± 0.8	162.9 ± 1.0	163.5 ± 1.0	164.2 ± 1.6
5/60	160.8 ± 1.2	159.4 ± 1.1	161.1 ± 0.9	162.2 ± 1.0
5/80	161.3 ± 1.3	162.5 ± 1.1	163.1 ± 0.9	164.5 ± 0.9
5/100	158.6 ± 1.3	157.8 ± 1.4	159.8 ± 0.8	162.5 ± 1.4
10/40	161.3 ± 1.2	160.4 ± 0.9	162.5 ± 0.9	165.3
10/60	160.8 ± 1.0	161.2 ± 1.1	160.2 ± 1.6	164.6 ± 1.5
10/80	162.1 ± 1.3	162.7 ± 1.4	163.2 ± 1.0	165.2 ± 1.2
10/100	160.3 ± 1.1	163.2 ± 1.0	164.6 ± 1.5	165.5 ± 1.4

Table 3. Rat survival result after ECT with dose factor

Dose (C)	Rat No.	Alive No.	Died of Lymph node	Died of primary tumor	Survival time (Day)
0	10	0	0	10	8.2 ± 0.86
40	30	0	2	28	19.7 ± 1.99
60	30	3	7	20	46.3 ± 8.42
80	30	5	21	4	67.4 ± 9.56
100	30	6	21	3	70.4 ± 9.55

Table 4. The percent 0f tumor necrosis after ECT

Tumor necrosis percent by spacing factor

Spacing (mm)	Rat No.	Tumor necrosis (%) 0	30	40	50	60	70	80	90	95	Mean	
5	40	6	0	4	10	4	6	6	3	1	54.1	
10	40	3	2	1	9	6	7	7	4	1	60.4	
15	40	3	0	4	5	12	6	8	2	0	59.2	
ALL	120	12	2	9	24	22	19	21	9	2	57.9	p-value = 0.552

Tumor necrosis percent by dose factor

Dose (C)	Rat No.	Tumor necrosis (%) 0	30	40	50	60	70	80	90	95	Mean	
10	30	8	2	4	7	3	4	2	0	0	39.7	
20	30	3	0	2	12	6	3	4	0	0	52.3	
40	30	1	0	3	4	9	6	5	2	0	62.0	
80	30	0	0	0	1	4	6	10	7	2	77.7	
ALL	120	12	2	9	24	22	19	21	9	2	57.9	p-value < 0.00001

Table 5. Summary of ECT-induced tumor necrosis overlap by spacing and dose

Spacing (mm)	N	No	Yes	Percent	Dose	N	No	Yes	Percent
5	40	7	33	82.5	10	30	10	20	66.7
10	40	6	34	85.0	20	30	8	22	73.3
15	40	14	26	65.0	40	30	7	23	76.7
					80	30	2	28	93.3
ALL	120	27	93	77.5	ALL	120	27	93	77.5
X-square = 5.45, p-value = 0.066					X-square = 6.64, p-value = 0.084				
Logistic regression: one-side p = 0.03					Logistic regression: one-side p = 0.0076				

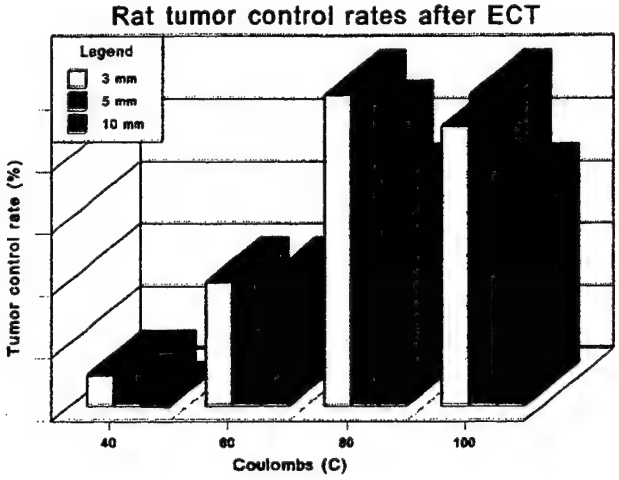
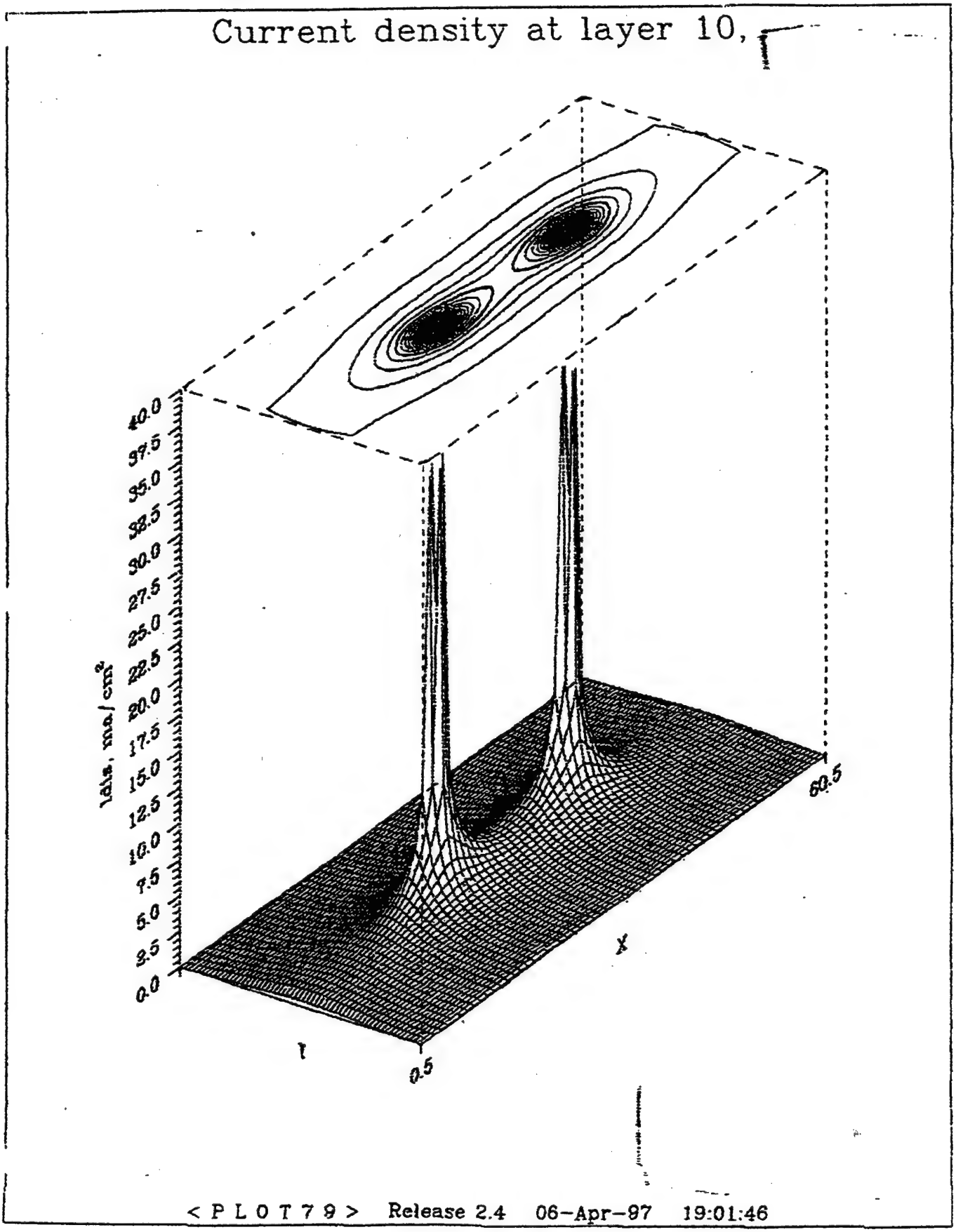


Figure 1. Rat tumor control rates after EChT

Figure 2. Current density pattern at layer 10



KEY RESEARCH ACCOMPLISHMENTS

1. The tissues around electrodes were necrosis after ECT, which indicated that ECT is effective for cellular disruption and death of the affected tissue.
2. Both anode and cathode can damage and destroy cell structures, which indicated that the cathodes should not be inserted into the normal tissues.
3. The effects of ECT are non-specific since it will destroy both tumor and normal tissues. Due to the non-specificity of its effect, all of the electrodes should be inserted into the tumor tissues.
3. With increasing dose, the tumor local control and rat survival rates increased. The spacing 3, 5, and 10 mm did not significantly affect the tumor responses to EChT if given the same dose.
4. It was difficult to perform EChT with 3 mm spacing. For a 2 x 2 x 2 cm^3 (approximately volume 5.2 ± 1 cm^3) rat breast cancer, single 80 C EChT with a 5 - 10 mm spacing could achieve satisfactory results.
5. Under microscope, as dose increased, the percent of the tumor necrosis and the necrosis overlap rate increased. There was a highly significant dose effect on the EChT induced tumor necrosis percent and necrosis overlapping rate. There was no significant spacing effect on the tumor necrosis percent and only weak evidence of a decrease in the rate of overlapping necrosis regions at the widest spacing.
6. Impedance method to calculate the current density during EChT showed that the high current density around the electrode was only limited at about 2 mm.

REPORTABLE OUTCOMES

1. Ren, R.L., N. Vora, W. W. Wang, J. R. Li, C. Staud, C. K. Chou, Variation of dose and electrode spacing for rat breast cancer electrochemical treatment. 21th Annual meeting of Bioelectromagnetics Society, Long Beach, California, June 20-24, 1999, p. 40.
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CONCLUSION

We have accomplished the specific aims as we proposed in our USAMRMC Breast Cancer Research Program grant. Through our studies, we concluded that:

(1) The tissues around electrodes become necrotic after EChT. The effects of EChT are non-specific since it destroys both tumor and normal tissues. Due to the non-specificity of its effect, the cathodes should not be inserted into the normal tissues and all of the electrodes should be inserted into the tumor tissues.

(2) In the survival study, with increasing dose, the local control and survival rates increased. The spacing 3, 5, and 10 mm did not significantly affect the tumor responses to EChT if given the same dose. However, it was difficult to perform EChT with 3 mm spacing. For a 2 x 2 x 2 cm^3 (approximately volume 5.2 ± 1 cm^3) rat breast cancer, single 80 C EChT with a 5 - 10 mm spacing could achieve satisfactory results.

(3) The results of the pathological study were consistent with the survival study. As dose increased, the percent of the tumor necrosis and the necrosis overlap rate increased. There was a highly significant dose effect on the EChT induced tumor necrosis percent and necrosis overlapping rate. There was no significant spacing effect on the tumor necrosis percent and only weak evidence of a decrease in the rate of overlapping necrosis regions at the widest spacing.

(4) Impedance method to calculate the current density during EChT showed that the high current density around the electrode was only limited at about 2 mm.

(5) For a diameter 2.0 to 2.5 cm rat breast cancer, EChT with 5 - 10 mm spacing and at least 80 C are the best parameter combinations.

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BIBLIOGRAPHY OF PUBLICATIONS AND MEETING ABSTRACTS

1. Chou, C.K. , N. Vora, J.R. Li, Y. Yen, R.L. Ren, J.A. McDougall, and B.S. Zhou. Development of electrochemical treatment at the City of Hope. 2nd World Congress for Electricity and Magnetism in Biology and Medicine, Bologna, Italy. June 8-13, 1997, p.99.
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LIST OF PERSONNEL RECEIVING PAY

C.K. Chou
J.A. McDougall
J.R. Li
R.L. Ren
W.W. Wang
Y. L. Xin

Second World Congress for Electricity and Magnetism in Biology and Medicine

ABSTRACT BOOK

The Bioelectromagnetics Society

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Palazzo della Cultura e dei Congressi
Bologna, Italy

C-11

DEVELOPMENT OF ELECTROCHEMICAL TREATMENT AT THE CITY OF HOPE. C.K. Chou, N. Vora, J.R. Li, Y. Yen, R.L. Ren, J.A. McDougall and B.S. Zhou. Division of Radiation Oncology and Department of Medical Oncology, City of Hope National Medical Center, Duarte, California 91010, USA.

At the 1st International Symposium on Electrochemotherapy of Cancer held October 20-23, 1992 in Beijing, Dr. Xin Yu-ling of the China-Japan Friendship Hospital reported 2516 patients results. Electrochemical treatment (ECT) of cancer utilizes direct current to induce chemical changes that kill cancer cells. A low-voltage direct current is maintained between anodes and cathodes in a tumor to deliver a dose of 100 coulombs per cubic centimeter. Cells near the electrodes cannot survive due to electrophoresis of positive and negative ions, electrolysis of tissue fluid, and electroosmosis of water. Clinical results suggest that ECT can be effectively used as a local therapy. This method is especially suitable for old and weak patients who are unable to endure surgery, or for patients who have failed radiation or chemotherapy. In addition, this alternative method is simple and economical. However, ECT is not a well established method, e.g. different electrode configurations and doses have been used. The mechanisms of ECT anti-tumor effect are not well understood. Systematic basic research is necessary before this method can be accepted for cancer treatment in the United States.

OBJECTIVE: To make electrochemical treatment a useful alternative method for treating solid tumors.

METHODS: The study at the City of Hope started with a seed grant from the Office of Alternative Medicine in September of 1993 to study the electrochemical treatment of fibrosarcoma in C3H/HeJ mice and Fisher 344 rats. Different electrode insertion configurations and dose levels were tested and tumor free cure rate was used as the end point. *In Vitro* studies were conducted on human KB oral cancer cells, which included: 1) cytotoxicity study exposing cells to different electrical doses, 2) clonogenic assay to study colony-forming abilities of the cells after ECT, 3) thymidine incorporation assay, and 4) pH measurement.

RESULTS AND DISCUSSION: All treated mouse and rat fibrosarcomas showed necrosis and regression; however, later tumor recurrence reduced long term survival. When multiple treatments were implemented, the best three-month mouse tumor cure rate was 59.3%, and the best six-month rat tumor cure rate was 75.0%. These preliminary results indicate that ECT is effective on mouse and rat fibrosarcomas. The effectiveness is dependent on electrode placement and dosage. These results have been published in 18(1), 1997 of Bioelectromagnetics. Using human KB cells *in vitro*, ECT was found to delay cell growth. ECT clearly demonstrates dose-dependent tumor cell growth inhibition by colony-forming assay. The toxicity effect may be due to disturbed DNA synthesis, resulting in decreased cell proliferation. Using our preliminary laboratory results and the reported clinical results from China as a basis, a clinical protocol "Phase I/II Study of Electrochemical Treatment of Recurrent Superficial Measurable Tumors" was submitted to the City of Hope institutional review board. For more than a year, the clinical protocol was reviewed and revised many times. Safety was the main concern since this treatment has not been reported in the United States. An investigational device exemption approval from the FDA resolved this concern. This approval is limited to one institution and 25 patients. Clinical study started on October 3, 1996 with the presence of Dr. Xin Yu-ling. Subsequently, more superficial tumor cases were treated. Initial results will be presented. Recently, we were funded by the Army Breast Cancer Research Program for two years to study ECT on rat breast cancer. The study is on 1) proper electrode placement, 2) optimal spacing and dosage, using MTF7 breast tumor on rats.

CONCLUSION: The results of these studies will help formulate a standardized ECT method for treating cancer patients and provide a better understanding of ECT mechanisms. A standardized method will enable physicians to treat those cancer patients who are untreatable with conventional therapies in a consistent and confident manner. The goal of this research is to make ECT a useful alternative method for treating localized cancer.

C-12

ELECTROCHEMOTHERAPY IN RESISTANCE TO cis-DIAMMINEDICHLOROPLATINUM(II) *IN VITRO* AND *IN VIVO* IN MICE. D. Miklavcic[1], M. Cemazar[2], B. Leon[3], L.M. Mir[3], J. Belehradek, Jr.[3], M. Bonnay[3], D. Fourcault[3] and S. Sersa[2]. [1]University of Ljubljana, Faculty of Electrical Engineering, 1000 Ljubljana, Slovenia. [2]Institute of Oncology, 1000 Ljubljana, Slovenia. [3]Institute Gustave-Roussy, 94805 Villejuif, France.

One of the ways to increase drug delivery into cells and tissues is by a local application of short intense electric pulses i.e. electropermeabilization. This approach is used in electrochemotherapy (ECT) to potentiate antitumor effectiveness of chemotherapeutic drugs. This study was performed in order to determine whether ECT can be used to potentiate CDDP antitumor effectiveness in resistant cells *in vitro* and *in vivo*. In sensitive TBL.Cl2 and resistant cells TBL.Cl2Pt IC50 doses were determined *in vitro* by colony forming ability test after chronic incubation with CDDP and were 0.05μg/ml and 0.46μg/ml, respectively. Thus, indicating Cl2Pt cells to be resistant to CDDP by a factor 10. Platinum content in Cl2 and Cl2Pt cells after 1 and 4 hours with CDDP at IC50 doses was comparable.

ECT was performed by placing 10E6 cells in suspension (50μl) with different CDDP concentrations between a pair of flat electrodes (distance 2mm) and applying 180V, 100μs, 8 electric pulses at repetition frequency 1Hz. After 5 minutes incubation cells were diluted 1000x, plated for colonies (1000 cells/p.d.) and IC50 doses were determined for both cell lines. The IC50 dose was 2.2 μg/ml for sensitive and resistant cells. Platinum content at CDDP doses 20μg/ml and higher was significantly increased after ECT in both cell lines as determined immediately after ECT or after 5 minutes.

IABC

PROCEEDINGS OF THE FOURTH INTERNATIONAL SYMPOSIUM ON BIOLOGICALLY CLOSED ELECTRIC CIRCUITS

SPONSORED BY THE INTERNATIONAL ASSOCIATION FOR BIOLOGICALLY CLOSED ELECTRIC CIRCUITS IN BIOMEDICINE (IABC)

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ELECTROCHEMICAL TREATMENT OF SUPERFICIAL TUMORS. C.K. Chou, N. Vora, J.R. Li, Y. Yen, R.L. Ren, Division of Radiation Oncology and Department of Medical Oncology, City of Hope National Medical Center, Duarte, California, 91010, U.S.A.

Electrochemical treatment (ECT) of cancer utilizes direct current to induce chemical changes that kill cancer cells. Clinical results suggest that ECT can be effectively used as a local therapy. This method is especially suitable for old and weak patients who are unable to endure surgery, or for patients who have failed radiation or chemotherapy. In addition, this alternative method is simple and economical. However, ECT is not a well established method. Systematic basic research is necessary before this method can be accepted for cancer treatment in the United States.

OBJECTIVE: To make ECT a useful alternative method for treating solid tumors.

METHODS: C3H/HeJ mice and Fisher 344 rats with fibrosarcoma were treated with ECT. Different electrode insertion configurations and dose levels were tested and tumor free cure rate was used as the end point. *In Vitro* studies were conducted on human KB oral cancer cells, which included: 1) cytotoxicity study exposing cells to different electrical doses, 2) clonogenic assay to study colony-forming abilities of the cells after ECT, 3) thymidine incorporation assay, and 4) pH measurement.

RESULTS AND DISCUSSION: All treated mouse and rat fibrosarcomas showed necrosis and regression; however, later tumor recurrence reduced long term survival. When multiple treatments were implemented, the best three-month mouse tumor cure rate was 59.3%, and the best six-month rat tumor cure rate was 75.0%. These preliminary results indicate that ECT is effective on mouse and rat fibrosarcomas. The effectiveness is dependent on electrode placement and dosage. Using human KB cells *in vitro*, ECT was found to delay cell growth. ECT clearly demonstrates dose-dependent tumor cell growth inhibition by colony-forming assay. The toxicity effect may be due to disturbed DNA synthesis, resulting in decreased cell proliferation. A clinical protocol "Phase I/II Study of Electrochemical Treatment of Recurrent Superficial Measurable Tumors" was approved by the Institutional Review Board and FDA. Clinical study started on October 3, 1996. Subsequently, more superficial tumor cases were treated. Initial results will be presented. Recently, we were funded by the Army Breast Cancer Research Program for two years to study ECT on rat breast cancer. The study is on 1) proper electrode placement, 2) optimal spacing and dosage, using MTF7 breast tumor on rats.

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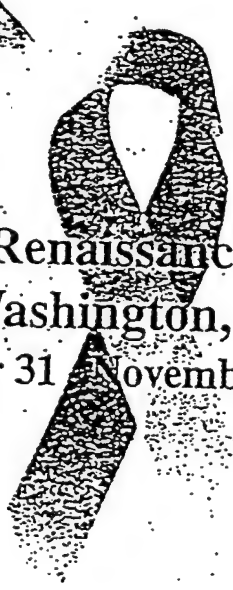
The Department of Defense
Breast Cancer Research Program Meeting

Era of Hope



The Renaissance Hotel
Washington, DC
October 31 - November 4, 1997

PROCEEDINGS, Volume III

ELECTROCHEMICAL TREATMENT OF RAT BREAST CANCER

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At the 1st International Symposium on Electrochemotherapy of Cancer held in Beijing during 1992, Dr. Xin Yu-ling of the China-Japan Friendship Hospital reported 2516 patients results. Electrochemical treatment (ECT) of cancer utilizes direct current to induce chemical changes that kill cancer cells. A low-voltage direct current is maintained between anodes and cathodes in a tumor to deliver a dose of 100 coulombs per cubic centimeter. Cells near the electrodes cannot survive due to electrophoresis of positively and negatively charged ions, electrolysis of tissues, and electroosmosis of water. Clinical results suggest that ECT can be an effective local cancer therapy. This method is especially suitable for older and weaker patients who are unable to endure surgery, or for patients who have failed radiation or chemotherapy. In addition, this alternative method is simple and economical. However, ECT is not a well established method, e.g. different electrode configurations and doses have been used. The mechanisms of ECT anti-tumor effect are not well understood. Systematic basic research is necessary before this method can be accepted for cancer treatment in the United States.

The City of Hope study started with a seed grant from the Office of Alternative Medicine of the NIH in September of 1993 to study the electrochemical treatment of fibrosarcoma in C3H/HeJ mice and Fisher 344 rats. Different electrode insertion configurations and dose levels were tested and tumor free cure was used as the end point. All treated mouse and rat fibrosarcomas showed necrosis and regression; however, later tumor recurrence reduced long term survival. When multiple treatments were implemented, the best three-month mouse tumor cure rate was 59.3%, and the best six-month rat tumor cure rate was 75.0%.

Keywords: Electrochemical Treatment, Breast Cancer, pH, Rat, Necrosis

This work was supported by the U.S. Army Medical Research and Matériel Command under DAMD17-96-1-6184.

These preliminary results indicate that ECT is effective on mouse and rat fibrosarcomas. The effectiveness is dependent on electrode placement and dosage.

In Vitro studies were conducted on human KB oral cancer cells, which included: 1) cytotoxicity study exposing cells to different electrical doses, 2) clonogenic assay to study colony-forming abilities of the cells after ECT, 3) thymidine incorporation assay, and 4) pH measurement. ECT was found to delay cell growth. ECT clearly demonstrates dose-dependent tumor cell growth inhibition by colony-forming assay. The toxicity effect may be due to disturbed DNA synthesis, resulting in decreased cell proliferation.

Under the Army support starting February 1, 1997, we are studying the effects of ECT on rat breast cancer. We examined the pathological changes in tumor samples removed post-treatment from Fisher 344 female rats injected with rat breast cancer cells. Forty eight rats were injected with 10^6 MTF-7 breast cancer cells. The tumors were allowed to grow to approximately 2 x 2 x 2 cm. Eleven rats were untreated due to premature necrosis of the tumor. Three rats in each group were treated with 40, 30, 20, 10 and 5 coulombs (C). Twelve rats were controls, receiving 0 C. A 4-channel BK29A ECT machine was used to administer the predetermined dosage. Two platinum needles were inserted at a 1-cm spacing into the tumor. One positive lead (anode) and one negative lead (cathode) were attached to the inserted platinum needles. The ECT device was configured with 8.0 V, a maximum of 80.0 mA, the charge determined by the treatment group, and a maximum treatment time of 3.0 hrs. Tumor samples were removed at 0, 24, and 48 hrs post-treatment and prepared for light microscopy and electron microscopy examination. The samples were examined for morphological changes.

Our observations indicate that there are distinct changes to the cellular structure of the treated tumors. The extent of cell structure disruption was related both to the condition of the tumor at the time of treatment and the dose applied during treatment. Preliminary results indicated changes ranging from blurred to complete loss of cellular outlines. An abrupt transition from treated tumor to viable tumor indicated that there is a definite effective treatment area, based upon the dose applied. The area adjacent to the cathode exhibited minor swelling, while dehydration was observed around the anode.

In some European studies, the cathodes have been placed in normal tissue. We examined the effects of placing both the cathode and anode in muscle and/or tumor tissues. Ten coulombs were applied. Tissue samples were obtained and prepared as previously. We observed disruption in the treated muscle tissue, either with cathode, anode, or both.

Based upon the results, we conclude that ECT is effective for cellular disruption and death of the affected tissue. Due to the non-specificity of its effect, the electrodes should be inserted in the tumor and not in normal tissue. The next step in our study will be determining the dose-response relationship and the optimal electrode position in the rat breast tumor. Spatial and temporal changes of pH in the tumor will be analyzed in detail.

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Electrochemical Treatment of Localized Tumors with Direct Current

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ABSTRACT: To develop cancer electrochemical treatment (ECT) in the United States, we have conducted basic studies and started a Phase I clinical trial. Our *in vivo* preliminary results indicate that ECT is effective on RIF-1 mouse tumor and rat fibrosarcomas. In the *in vitro* study ECT clearly demonstrated dose-dependent human oral carcinoma KB cell growth inhibition by colony-forming assay. We have started an animal study to resolve the ECT methodological problems for rat breast cancer. Meanwhile, we began a clinical trial treating patients with recurrent superficial measurable malignant tumors. Our goal is to make ECT a useful alternative method for treating localized tumors.

INTRODUCTION:

Electrochemical treatment (ECT) of cancer has been used for only a few decades. Since Nordenström reported his clinical results [1], this method has been studied in Japan, Slovenia, and China [2,3,4]. Xin [4] modified Nordenström's original method by inserting both cathode and anode platinum electrodes into tumors of conscious patients. A constant voltage of less than 10 V is applied to produce a 40-80 mA current between the anodes and cathodes for 30 minutes to several hours, delivering 100 coulombs per cubic centimeter. Due to electrolysis, electrophoresis, and electroosmosis, cells near the electrodes are killed by the microenvironmental changes [5].

Although a number of clinical studies have shown that ECT has an antitumor effect and has been used as an alternative method for cancer treatment, ECT has not been universally accepted. The reason is that the published data lacks essential preclinical studies, and reliable controlled clinical trial is missing. It is necessary to conduct preclinical studies and clinical trials to verify its value. In this paper, we outline our efforts during the last five years in developing ECT as an alternative cancer treatment at the City of Hope.

DEVELOPMENT:

Office of Alternative Medicine Grant

The ECT study at the City of Hope started in September of 1993 with a seed grant from the National Institute of Health, Office of Alternative Medicine. The specific aims were to conduct an *in vivo* study to standardize animal experiments, to establish dose-response relationship, and to develop a large scale study proposal.

In vivo studies were conducted to evaluate the effectiveness of ECT on animal tumor models and to standardize animal experiments [6]. Radiation-induced fibrosarcomas were subcutaneously implanted in 157 female C3H/HeJ mice. Larger rat fibrosarcomas were implanted on 34 female Fisher 344 rats. When the spheroidal tumors reached 10 mm in the mice, 2-5 platinum electrodes were inserted into the tumors at various spacings and orientations. Ten rats in a pilot group were treated when their ellipsoidal tumors were about 25 mm long; electrode insertion was similar to the later part of the mouse study, i.e., two at the base and two at the center. A second group of 24 rats were treated with 6-7 electrodes when their tumors were about 20 mm long, all electrodes were inserted at the tumor base. Of the 24 rats, twelve of these were treated once, ten treated twice, and two treated thrice. All treated tumors showed necrosis and regression for both mice and rats; however, later tumor recurrence reduced long term survival. When multiple treatments were implemented, the best three-month mouse tumor cure rate was 59.3%, and the best six-month rat tumor cure rate was 75.0%. These preliminary results indicate that ECT is effective on the RIF-1 mouse tumor and rat fibrosarcoma. The effectiveness is dependent on electrode placement and dosage.

In Vitro studies were conducted on human KB oral cancer cells [7], which included: 1) cytotoxicity study exposing cells to different electrical doses, 2) clonogenic assay to study colony-forming abilities of the cells after ECT, 3) thymidine incorporation assay, 4) pH measurement. ECT was found to delay cell growth by using 1.5 coulomb (400 μA x 75 min) in 5 ml of culture medium. ECT clearly demonstrates dose-dependent tumor cell survival by colony-forming assay. Cytotoxicity study by methylene blue assay determined that the median toxic concentration (ID_{50}) value is 3 coulomb (400 μA x 125 min)/5 ml to 2.5 x 10^5 cells in culture. For a fixed dose (3 coulomb, 400 μA x 125 min/5ml), the higher the current, the less cell kill due to shorter treatment time. Time is, therefore, an important factor. When cell concentration was altered, the survival was higher for increased cell concentrations. Thymidine incorporation assay indicated that ^3H thymidine incorporation into DNA decreases as ECT dose increases. At 5 coulomb (400 μA x 125 min)/5

ml, pH at the anode decreased to 4.53 and at the cathode increased to 10.46. These results indicate that ECT is effective for killing human KB cancer cells in culture system and the toxicity effect is related with coulomb, i.e., current and treatment time.

Army Breast Cancer Research Program

The most important specific aim of the Office of Alternative Medicine's seed grant was to develop a large-scale study proposal. Breast cancer is a leading cause of women cancer death. Furthermore, the local regional recurrence following standard treatment is one of the major problems for patients with breast cancer. Effective local control is an unsolved problem. In order to make ECT available for breast cancer patients in the United States, who have failed or cannot bear conventional therapy, we developed a research proposal to resolve the ECT methodological problems for breast cancer. This research proposal, entitled "Electrochemical Treatment of Breast Cancer with Direct Current", is being funded for two years by the Department of Defense Breast Cancer Research Program, USAMRMC (United States Army Medical Research and Matériel Command).

This research project was proposed to resolve the ECT methodological problems. The MTF7 rat breast cancer cell line implanted in Fisher 344 rats' mammary fat pads will be used as a cancer model. We will test the hypothesis that tumor responses are dependent on electrode spacing and treatment dose, and will determine the optimal spacing and dose for treating breast cancers. Morphology of the tumors treated with various electrode spacings and doses will be tested. This morphology study will determine the effective region. Finally, tumor size, local control rate, and rat survival rate with various electrode spacing and doses will be studied. This hypothesis will provide essential information about optimal electrodes spacing and dosage. Division of Anatomic Pathology has participated in this study. The results of this research will help formulate a standardized ECT method for breast cancer treatment and provide a better understanding of ECT mechanisms.

Phase I Clinical Trial

Besides the basic research program, we have also started a clinical trial entitled "Phase I study of electrochemical treatment of recurrent superficial measurable malignant tumors." A clinical protocol was submitted to the institutional review board (IRB) at the City of Hope in June 1995. For more than a year, the clinical protocol was reviewed and revised many times. Safety was the main concern since this treatment has not been reported in the United States. According to an FDA investigational device exemption (IDE) regulation, ECT was considered as a significant risk device. Therefore, an application to FDA was submitted in April 1996, and an approval was granted in July 1996. This protocol was approved by the IRB in September 1996. The first patient treatment took place on October 3, 1996.

The Phase I trial asks for 25 recurrent superficial measurable malignant tumors treatment. The purposes of these clinical trials are to evaluate ECT tumor response and record the acute and late ECT toxicities in the treatment of superficial tumors. Five late stage patients have been treated as of July 1997. Three patients developed complete response, one partial response, and one no response (tumor reduced less than 50%) due to incomplete treatment. All patients tolerated treatment well.

CONCLUSIONS:

During the past five years, ECT has been developing at the City of Hope. Our preliminary *In Vitro* and *In Vivo* studies indicated that ECT is an effective tumor treatment. It confirmed the results reported in other studies, and through these efforts, we were able to start ECT clinical trials in the United States. It could be expected that our research program, including basic researches and clinical trials, will help formulate a standardized ECT method for cancer treatment and provide a better understanding of ECT mechanisms. The goal of this research is to make ECT a useful alternative method for treating localized tumors.

ACKNOWLEDGMENTS:

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μseç. The bursts were supplied with frequencies of 0.1 Hz, or one burst per 10 sec. The rats were stimulated by the train-pulsed magnetic fields for intervals of 1000 sec, 2000 sec, and 3000 sec. The coil had a liquid cooling system, and the coil temperature was constant at 22 °C. Each rat in the M(-) group, and not exposed to magnetic fields, was in an acrylic capsule, meanwhile, each rat in the M(+) group was exposed to magnetic fields. The rats of the control group were neither exposed to magnetic fields, nor in the acrylic capsule. Some rats in each of the three groups were injected with Evance Blue (EB) into their femoral vein 1hour after stimulation. One hour after injection, the rats injected with EB were sacrificed by perfusion-fixation of the brain. The EB in the brain was investigated both macroscopically and optical microscopically. Some were injected with the horseradish peroxidase (HRP). The HRP in the brain was investigated using optical and electron microscope. Others were sacrificed by perfusion-fixation of the brains 1hour after stimulation and the albumin in the brain was demonstrated by immunohistochemistry.
RESULTS: In three groups, the EB or the HRP in the brain were not detected macroscopically or microscopically. In addition, and the albumin was not detected in the brain using immunohistochemistry.
DISCUSSION: The permeability of the BBB induced by microwave was reported, but thermal effects caused most of the results. This study focused on the non-thermal effects of exposure of electromagnetic fields by using coils with a liquid cooling system and pulsed magnetic stimulation.
CONCLUSION: Repetitive pulsed magnetic stimulation with a pulse-width of 200 μsec at 85mT for a period of less than 3000sec does not affect the permeability of the blood-brain barrier.

P-52B

ELECTROCHEMICAL TREATMENT INDUCES NECROSIS OF RAT BREAST CANCER. J.R. Li, W.W. Wang*, L.M. Weiss*, J.D. Hardy*, H. Lee*, J.A. McDougall, R.L. Ren*, N. Vora* and C.K. Chou. Department of Radiation Research, Division of Pathology, and Department of Research Resources, City of Hope National Medical Center, Duarte, California 91010, USA.

Electrochemical treatment (ECT) of cancer is a promising new method which delivers direct current (DC) into tumor tissue by inserting electrodes (anodes and/or cathodes) to induce tumor regression. Dr. Björn Nordenström of Sweden was the first in recent years to utilize DC for treating human lung tumors. This method was later used to treat breast cancer. The most impressive data, consisting of 2516 cases from 66 hospitals in China, were reported by Dr. Xin Yu-ling. ECT has become an alternative method for cancer treatment in China with more than 10,000 patients treated. Even though ECT has been applied in clinical studies of different kinds of tumors and has shown positive responses, there has been almost no systemic research in this field. Little has been done on the basic research, such as effectiveness evaluation, dose-response relationship, and cytotoxicity. The biological mechanism of ECT is also poorly understood. Also, the treatment methods are arbitrarily. For instance, in some European studies, the cathodes have been placed in normal tissue.
OBJECTIVE: To evaluate the effectiveness of ECT by investigating the pathological changes of rat breast cancer tissue and normal muscle tissue.
METHODS: (1)Tumor tissue responses to ECT: Rat breast cancer cells (MTF-7) were maintained and grown in Minimum Essential Modified Eagle's Medium with Eagle's Salts and L-Glutamine (MEM) supplemented with 10% heat-inactivated fetal bovine serum and penicillin (100 U/ml)-streptomycin (100 mg/ml) solution with 5% CO_2 in a 37 °C incubator. One million cells grown in logarithmic phase were injected subcutaneously into the right fat pad of the mammary gland of Fisher 344 female rats. While the tumors were grown to approximately 2×2×2 cm, 54 rats were divided into 6 groups (9 in each group) and underwent ECT with doses of 0, 5, 10, 20, 30, and 40 coulombs, respectively. A 4-channel BK29A ECT machine was used to administer the predetermined dosage. Two platinum needles were inserted at a 1-cm spacing into the tumor. The ECT device was configured with 8.0 V, a maximum of 80.0 mA, the charge determined by the treatment group, and a maximum treatment time of 3.0 hrs. Three tumor samples in each group were removed at 0, 24, and 48 hrs post-treatment and prepared for light microscopy and electron microscopy

examination. The samples were examined for morphological changes. (2) Normal muscle tissue response to ECT: Nine Fisher 344 female rats were used and 10 coulombs were applied in right thigh muscle. Treatment methods and tissue sample preparation were the same as the tumor study.

RESULTS: There are distinct changes to the cellular structure of the treated tumors. The extent of cell structure disruption was related both to the condition of the tumor at the time of treatment and the dose applied during treatment. Preliminary results indicated changes ranging from blurred to complete loss of cellular outlines. An abrupt transition from treated tumor to viable tumor indicated that there is a definite effective treatment area, based upon the dose applied. The area adjacent to the cathode exhibited minor swelling, while dehydration was observed around the anode. Disruptions were also seen in the treated muscle, either near cathode or anode.

CONCLUSION: Electrochemical treatment is effective for cellular disruption and death of the affected tissue. Due to the non-specificity of its effect, the electrodes should be inserted in the tumor and not in normal tissue.

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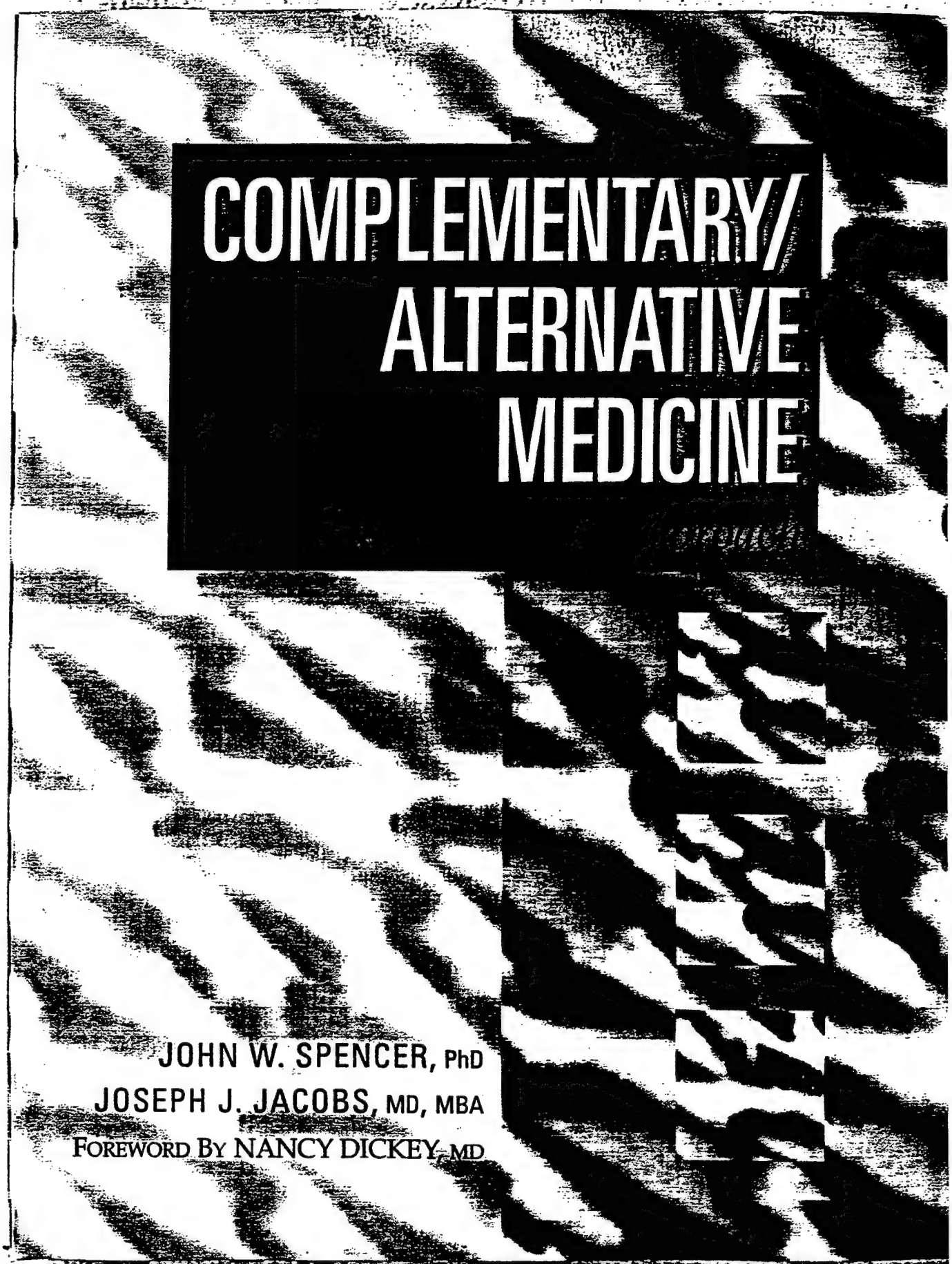
P-53A

TERATOGENIC AND DEVELOPMENTAL EFFECTS OF LOW FREQUENCY MAGNETIC FIELDS. J. Juutilainen. Department of Environmental Sciences, University of Kuopio, 70211 Kuopio, Finland.

A research program consisting of several experimental and epidemiological studies was conducted to investigate the possible adverse effects of ELF or VLF magnetic fields on pregnancy outcome. Some of the results have been published. This presentation reports recent unpublished observations and discusses the results of the entire research program. Several animal studies were conducted using Wistar rats and two strains of mice (CBA/Ca and CBA/S). Pregnant females were exposed for the entire pregnancy to 50 Hz sinusoidal magnetic fields at 13 or 130 μT (r.m.s.), or to 20 kHz magnetic fields with a sawtooth waveform and peak-to-peak flux density of 15 μT. Mouse embryos were exposed *in vitro* to 50 Hz, 13 μT sinusoidal fields. No increase of gross malformations was found in the exposed animals, and most of the other variables measured were not affected by magnetic field exposure. However, all magnetic field exposures tested (50 Hz and 20 kHz, all flux densities) were associated with significantly increased minor skeleton anomalies in Wistar rats and CBA/Ca mice. The experiments with CBA/S mice provided some support for earlier studies suggesting increased fetal loss (resorptions) in the exposed animals, but no such effect was seen in CBA/Ca. In epidemiological studies, a suggestive association was found between early pregnancy loss and residential exposure to 50 Hz magnetic fields above 0.6 μT. Further studies have investigated possible association of residential magnetic fields (assessed by short-term measurements) with pregnancy delay and low birth weight. The combined experimental and epidemiological results do not suggest strong effects on pregnancy outcome. However, weak biological effects on embryonic development possibly exist. These effects may be modified by other external and internal (genetic) factors that interact with magnetic field exposure. Existence of biological effects does not necessarily mean that there are adverse effects. Adverse effects in some conditions at high field strengths cannot be excluded based on current evidence.


COMPLEMENTARY/
ALTERNATIVE
MEDICINE
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COMPLEMENTARY/ ALTERNATIVE MEDICINE

An Evidence-Based Approach

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CHAPTER 2

Preclinical Studies in Complementary/ Alternative Medicine

Chung-Kwang Chou and Ru-Long Ren

Preclinical studies are important for complementary/alternative medicine (CAM) and may act as a bridge to the understanding of findings subsequently developed from clinical studies. First, CAM includes lifestyle practices, clinical tests, or therapeutic modalities that are generally promoted for the prevention, diagnosis, or treatment of diseases.[5,18] Therefore determination of their safety and effectiveness should be considered a priority. When their efficacy and safety are proven through detailed preclinical studies, including in vitro, in vivo, and clinical trials, CAM therapies can become a part of mainstream medicine, and as long as the efficacy and safety of such treatments remain unproved, those methods and agents should be abandoned to save money and avoid unnecessary harm to the public. For example, Laetrile (amygdalin) was thought to have anticancer effects. It achieved great popularity in the 1970s and was eventually legalized for use in 27 states. Public interest resulted in a National Cancer Institute–supported study that showed no effect for Laetrile against cancer.[37]

Second, some CAM treatments have already been used in clinics as effective methods, but due to lack of systemic preclinical studies, their optimal method may not be established and their maximum effects may not be achieved. For example, electrochemical treatment (ECT) for cancer has been used in China and Europe in thousands of patients. It has been shown that ECT is an effective, safe alternative therapy for some cancers.[31,41,59,60] However, ECT is not a well-established method. We believe that if an optimal method is established and the mechanisms of ECT cell death are understood through a number of preclinical studies, ECT will provide a more effective and understandable alternative therapy for some localized cancers.

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The major objective of this chapter is to describe the development of certain preclinical studies in CAM. We define preclinical studies as basic research and clinical trials (Phases I to III). First, we give an overview of preclinical evaluations in conventional medicine; then we describe ECT and hyperthermia to illustrate the progression from basic research to clinical trials.

STEPS OF PRECLINICAL EVALUATIONS IN CONVENTIONAL MEDICINE

In most countries tests of drugs and medical devices are regulated by legislation and closely monitored by government agencies. In the United States federal consumer protection laws require that drugs and devices used for the prevention, diagnosis, or treatment of disease be demonstrated as both safe and effective before being marketed.[35] To meet these requirements, preclinical and clinical evaluations of a new treatment must undergo a number of steps in the evaluation of its potential effectiveness and safety. The regulatory agencies also require full disclosure of how products are manufactured and devices are designed, and how they function. In this section we describe the process of the discovery, development, and regulation of a new drug.*

The process of discovering and developing a drug involves substantial time, effort, and resources. Berkowitz and Katzung[3] and Grever and Chabner[20] have described the procedures. The first step in the development of a new drug is to discover or synthesize a potential new drug molecule. Most new drug candidates are identified through empiric, random screening of biologic activity of a large number of natural products as a result of rational chemical modification of a known molecule, or by designing a molecule based on an understanding of biologic mechanisms and chemical structure. A variety of biologic in vitro and in vivo assays (at the molecular, cell, organ, and whole animal levels) are used to define the activity and selectivity of the drug. Subsequently, through these studies more potent, less toxic derivatives often can be developed.

The second step in the development of a new drug is to conduct pharmacologic studies that include safety and toxicity tests. Candidate drugs that survive the initial screening and profiling procedures must be carefully evaluated for potential risks before beginning clinical testing. Preclinical toxicology is frequently the third step in the progression of a new drug from discovery to initial Phase I clinical trials in humans. The major kinds of preclinical toxicologic studies include (1) acute toxicity; (2) subacute and chronic toxicity; (3) effects on reproductive functions, including teratogenicity; (4) carcinogenicity; and (5) mutagenicity.[3,20,21,54] The major objectives of the preclinical toxicologic studies include the definition of the qualitative and quantitative organ toxicities, the reversibility of these effects, and the initial safe starting dose proposed for humans. Several quantitative estimates are desirable. These include the "no-effect" dose, which is the smallest dose that is observed to kill any

*References 1, 3, 9, 20, 32, 62.

animal, and the median lethal dose, which is the dose that kills approximately 50% of the animals.

It is important to recognize the limitations of preclinical testing. There is no guarantee that the human subject will accommodate a new drug in the same way as an animal species. Extrapolation of toxicity data from animals to humans is not completely reliable. For any given compound, the total toxicity data from all species have a very high predictive value for its toxicity in humans; however, there are limitations on the amount of information that is practical to obtain. In addition, since large numbers of animals are needed to obtain preclinical data, toxicity testing is time-consuming and expensive; for statistical reasons, rare adverse effects are unlikely to be detected, just as in clinical trials.

The fourth step in the development of a new drug is human evaluation—testing in humans begins after sufficient acute and subacute animal toxicity studies have been completed. Chronic safety testing in animals is usually conducted concurrent with clinical trials. Evaluation in humans includes three formal phases of clinical trials. Only after showing positive Phase III results on efficacy and safety can the new drug be permitted to be marketed for further postmarketing surveillance.[1,3,52]

The objective of *Phase I trials* is to determine a dose that is appropriate for use in Phase II trials. There are several different types of Phase I trials.[52] These trials are nonblind, or "open." A small number of healthy volunteers or patients with advanced disease resistant to standard therapy are included in such trials. However, it is important that the patients have normal organ functions because important pharmacokinetic parameters, such as absorption of drugs, the half-life of maximum tolerated dose, and metabolism, are often determined in this phase of trials. Many predictable toxicities are detected. The effects of the drug as a function of dosage are established in Phase I trials. It should be pointed out that because of the small sample size of Phase I trials, the pharmacokinetics parameters are generally determined imprecisely.

In *Phase II trials* the treatment is normally disease-type specific, and the aim is to identify the disease types suitable for treatment. The drug is studied for the first time in patients with the target disease to determine the efficacy of the drug. A broader range of toxicities may be detected in this phase. A small number of patients are studied in great detail. A single-blind design is often used.

Phase III is the controlled clinical trial in which the new treatment will be compared with a conventional therapy. Based on the information gathered in Phases I and II, this trial will further establish drug safety and efficacy in a much larger number of patients. Certain toxic effects, especially those caused by sensitization, may become apparent for the first time during this phase. Double-blind and crossover techniques are frequently employed. Phase III studies can be difficult to design and execute and are usually expensive, because of the large numbers of patients involved and the massive amount of data that must be collected and analyzed.

ELECTROCHEMICAL TREATMENT

Electrochemical treatment of cancer involves inserting platinum electrodes into tumors of conscious patients. A constant voltage of less than 10 V is applied to produce a 40- to 80-mA current between the anodes and cathodes for 30 minutes to several hours, delivering 100 coulombs (C) per cubic centimeter. As a result of electrolysis, electrophoresis, and electroosmosis, cells near the electrodes are killed by the microenvironmental changes. During the last 4 years we have been studying this method at the City of Hope National Medical Center in Duarte, California.

Nordenström[40-42] was the first in recent years to use direct current (DC) for the treatment of human tumors. He treated 26 lung metastases in 20 patients. Twelve of the 26 metastases regressed totally. Two patients with 5 of the 26 tumors were still alive 10 years later. In Japan, Nakayama[38] and Matsushima and colleagues[30] have treated human cancer with ECT combined with chemotherapy and radiation. Matsushima and co-workers[31] summarized 26 cases treated with ECT, including two cases of breast cancer (the majority of the 26 cases were inoperable because of poor general condition of the patient or an advanced cancer stage). There was improvement of symptoms (pain relief) in almost 50% of the patients. A decrease in tumor size to some degree was observed in 21 measurable lesions, two tumors disappeared completely, and no tumor cells remained in one case in which a histopathologic examination was performed. These results showed the usefulness of ECT alone to treat tumors, since the two tumors that completely disappeared had not responded to other, previous treatments. The main complication was pain during treatment; however, this complication spontaneously disappeared and did not require specific treatments.

In 1987, Nordenström introduced ECT to China. Because of the large patient load, authority of physicians, and minimal legal considerations in China, physicians were able to use ECT on thousands of patients with various kinds of cancers.[59] Xin[60] summarized the results of 2516 cases on 23 types of tumors. The primary cases were cancer of the lung (593 cases), the liver (388), the skin (366), and the breast (228). The 5-year survival rates were 31.7%, 17%, 67%, and 62.7%, respectively. In addition, ECT produces minimum trauma as compared with surgery. Also, unlike radiation therapy or chemotherapy, there are no serious side effects, and treatment can be repeated. Chinese physicians concluded that ECT is a simple, effective local therapy. The local control rates were considered satisfactory compared with those of conventional therapy. This method has been approved by the Chinese Ministry of Public Health and is used in approximately 1000 hospitals.[59]

Preclinical Issues

Although ECT is already prevalent in clinical practice in China and a number of clinical studies have shown that ECT has an antitumor effect, ECT has not been widely accepted in world clinics. The reason is that ECT is not a well-established method. The

data lack essential preclinical studies, and reliable control of the clinical trials is missing. Review of literature pertaining to this topic shows that precise guidelines regarding electrode insertion and electrical parameters (i.e., voltage, current, treatment duration) are not available. Therefore ECT can be a feasible treatment for some cancer patients, but we are not certain that the method presently used has achieved its optimal effectiveness. More basic research to address preclinical issues is necessary before ECT can be used for patients in the United States. Compared with the steps of preclinical evaluation of conventional medicine, the following research priorities must be given to ECT preclinical evaluation.

Methodology Studies

To make ECT a reliable, effective method for treating cancers, a standardized treatment must be determined. At this time, published clinical studies have shown various electrode insertion methods and distributions; different electrode placements have been used in Europe and China. Optimal electrode distribution has not been determined.

Electrode Insertion. Nordenström[41] of Sweden introduced the biologically closed electric-circuit (BCEC) concept. He named it the third circuit in the human body, after cardiovascular and lymphatic circuits. The BCEC circulates through the vascular interstitial closed circuit (VICC). In Nordenström's view[43] ECT is an artificial activation of the BCEC through the VICC. The flow of ionic current triggers interactions between the induced electromagnetic fields and the cancer tissue. Therefore Nordenström has been treating his lung cancer patients with the anode inserted in the tumor center and cathode in normal tissue several centimeters away from the tumor boundary. European researchers and physicians have followed this method. In China Xin and colleagues[61] modified the technique, putting both anode and cathode into the tumor, with the anode in the center and cathodes in the periphery. Chinese physicians have found that placing both the anodes and cathodes in tumors not only protected the normal tissue but also had a greater effect on the tumor.

To resolve the discrepancies of whether both the anode and cathode should be inserted within the tumor or whether the cathode should be in normal tissue, a detailed animal study should be conducted to test whether induced cell damage occurs around the anode and cathode during ECT. We believe that the morphologic responses of the ECT-treated cells near the anode and cathode should be studied to understand their pathologic mechanism. By comparing the cellular changes in tumors with those of normal muscles after ECT, this preclinical study would determine whether the placement of the cathode in normal tissue can cause problems.

Electrode Configuration. Another important preclinical issue in the study of ECT is electrode configuration. The studies described earlier would answer the question of

where the electrodes should be inserted and how many electrodes should be used. After several thousand patient treatments, Chinese physicians found that, for superficial tumors, if the electrodes were vertically inserted into the tumor, there were many cases of recurrence at the base of the tumor because the electric field generated at the tip is too small to destroy cells at the base of the tumor. However, by horizontally inserting an adequate number of electrodes at the tumor base, much better results were produced. We also observed this phenomenon in our animal studies.[14] Our preliminary mouse study,[14] using either two or five vertical electrodes or two horizontal electrodes going through the central part of the tumor, did not produce a high cure rate (Figs. 2-1 and 2-2). Later, in our rat study we adapted the tumor base method by inserting either six or seven electrodes at the tumor base perpendicular to the long axis of the tumor. Eighteen of 24 rats were cured for more than 6 months (Figs. 2-3 and 2-4). From the Chinese clinical experience and our animal results, we believe that inserting electrodes at the base of the tumor is the best method of ECT treatment of superficial tumors.

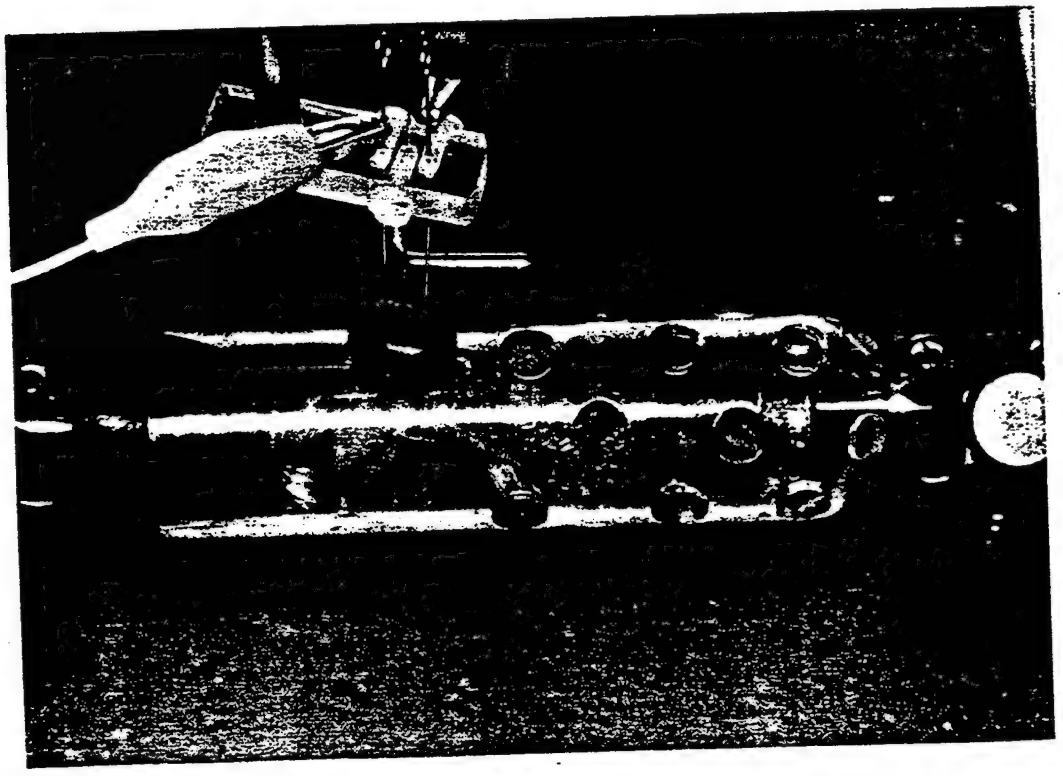

Fig. 2-1 Two platinum electrodes were vertically inserted into C3H/HeJ mice RIF-1 tumor at a spacing between 4 and 10 mm, depending on tumor size. A thermocouple for temperature measurement was in the central position.

Spacing. How many electrodes should be used? This depends on the tumor size and nature. Since the effective volume of the treatment is limited to the vicinity of the electrodes, it was thought that to cover the tumor region with an adequate number of electrodes is essential. In China it was reported that the effective volume around each electrode is about 3 cm in diameter; therefore spacing between electrodes is usually kept at less than 2 cm. However, it is not known for what type of tumor this range is effective. Japanese practitioners have used 3- to 4-cm spacing, while in Slovenia, the cathode (not the anode) was inserted in the skin tumor and a plate electrode was pasted on the skin at 3 to 4 cm from the edge of the melanoma skin lesion.[47] Since each cancer tumor has its own tissue conductivity, the effective volume differs for each different tumor; therefore the tissue conductivity must be determined. Rat tumor morphologic changes after ECT can be used to study the effect of spacing.

Preclinical Safety and Toxicity Test

Although ECT is already clinically prevalent in China and Chinese physicians have concluded that ECT is a simple, effective local therapy, the dosage guidelines have not been determined. To conduct preclinical safety and toxicity tests, priority studies should focus on dosage guidelines. Excessive dosage will cause pain, burns, and slow

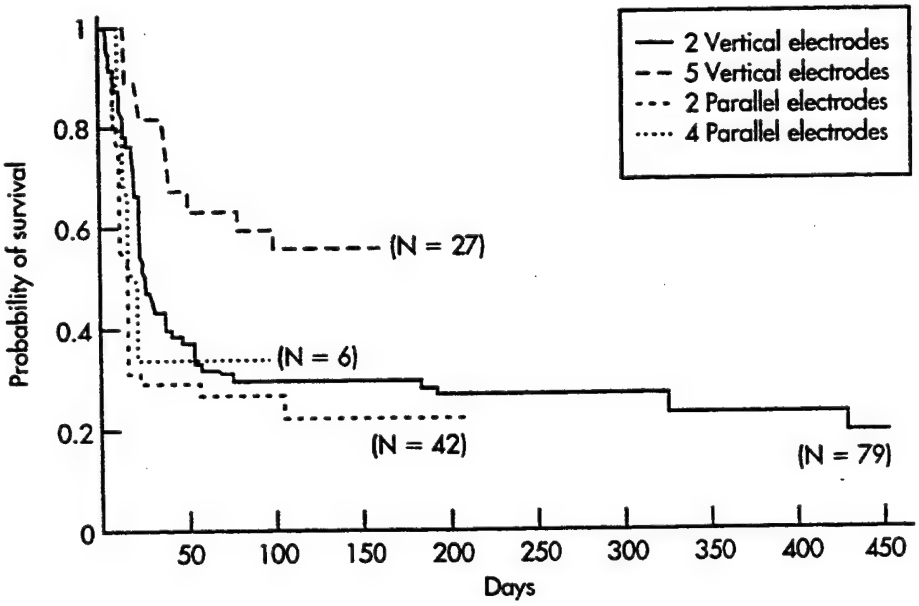

Fig. 2-2 Electrochemical treatment survival curves showing results for four groups of C3H/HeJ mice. The best results were with four electrodes inserted parallel to the body. (Modified from Chou CK et al: *Bioelectromagnetics* 18:14, 1997.)

Fig. 2-3 Electrodes were inserted at the base of the Fisher 344 rat fibrosarcoma. Insertion was perpendicular to the long axis of the tumor, and an arrangement of alternating cathodes and anodes was used.

healing. Inadequate dosage will result in ineffective treatment, which is life threatening for cancer patients. Therefore proper dosage is essential for ECT. Electrical dosage in tumors varies with many factors, such as tissue conductivity and electrode configuration. Each of these factors should be examined in detail.

Dosage. Nordenström[41] stated that "because few indications existed to guide an optimal choice of voltage and amount of electric energy to be given, an arbitrary amount of current of 100 coulombs per centimeter of tumor diameter (at 10 V potential) was chosen to be the preliminary dose." Xin[60] treated his patients with 40 to 80 mA current, 8 to 10 V, at 100 C/cm^3. The 100 C/cm^3 value is more appropriate than the per-centimeter-diameter value, since the dose should be related to the volume, not the diameter, of the tumor. Although 100 C has been used widely, the dosage guidelines used by Nordenström and Xin are arbitrary and there is no scientific basis for these values.

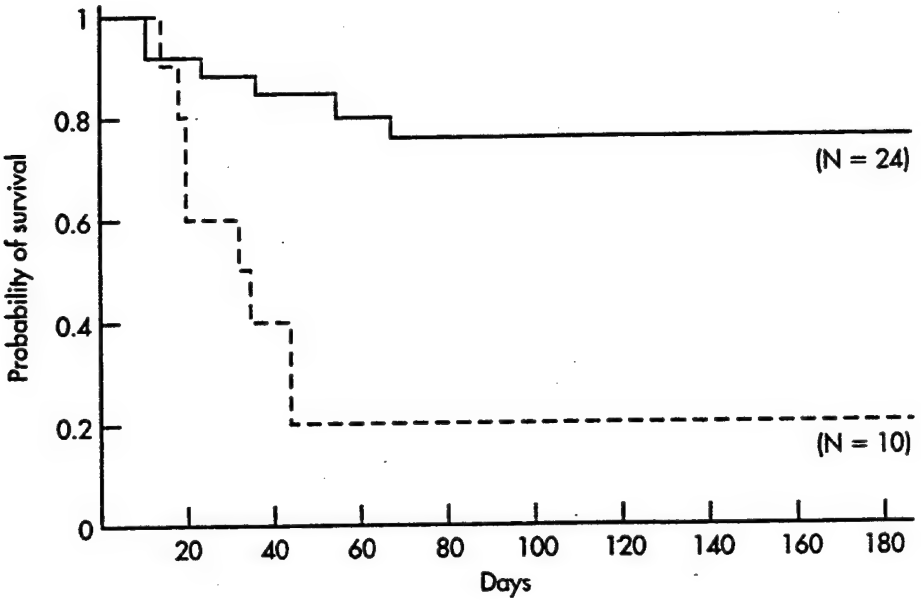

Fig. 2-4 Survival probability curves of the two groups of Fisher 344 rats that received electrochemical treatment. Ten rats were treated with two arrays of electrodes, and 24 rats were treated with multiple electrodes at the tumor base. The difference in survival was significant (log-rank test; $p = 0.001$). (Modified from Chou CK et al: *Bioelectromagnetics* 18:14, 1997.)

Electrical dose (coulombs) is a product of current (A) and time (sec). Higher current reduces treatment time but also causes pain. Lower current prolongs the treatment. Therefore a compromise with acceptable current density over a reasonable time is desirable. However, notable is the arbitrary definition of dose in coulombs per cubic centimeter. From the data published in China, apparently the charge is obtained by multiplying the DC passed between two electrodes by the time the current is applied, and dividing by the volume of the tumor. Clearly, the charge density is not uniform throughout the treated tumors. The charge density (i.e., dose distribution) is not appreciated. Many factors may affect the dose distribution. The charge density could be the source of the varying results when needles are implanted parallel or perpendicular to the body surface, or when changes in the numbers of needles and needle spacing are made. It would be advantageous to design dose experiments where one would be dealing with uniform charge density distribution rather than the highly nonuniform distributions obtained with needle electrodes. The lack of dosage guidelines has become a bottleneck in ECT development.

Safety and Toxicity. Electrochemical treatment must be carefully evaluated for potential risks before clinical use. Unlike chemical agents, ECT is a local therapy and no for-

eign agent is injected into the body. We can ignore some toxicity tests, such as effects on reproductive functions, including teratogenicity, carcinogenicity, and mutagenesis.

Based on published clinical data, ECT is used mainly in the treatment of patients who are not candidates for conventional therapy because of age or overall medical condition, or both. Compared with surgery, ECT is less traumatic; therefore recovery is quicker. There are no serious side effects from ECT as compared with radiation therapy or chemotherapy. However, since ECT destroys both normal and tumor tissue, there is a potential risk, depending on patient sensitivity, for certain parameters (e.g., voltage, current) to influence either quality or quantity of treatment. To bridge the gap between the animal studies and clinical evaluation, Phase I clinical trial is necessary. The following risks should be documented in preclinical tests:

- Local pain is the main acute complication during electrode insertion and ECT procedure.
- Tumor necrosis and ulceration are usually observed when superficial lesions are treated with ECT. The absorption of necrosis tissues may cause fever and leukocytosis after ECT.
- If the skin is not well insulated, it can be burned by the chemical reaction. The healing time depends on the size and location of the lesion. Platinum electrode bases should be insulated to prevent skin injury at the entrance site.
- During electrode insertion, blood vessels and nerves may be punctured. Therefore bleeding and pain may be observed during electrode insertion.

Mechanism Studies

Besides methodology and dosage studies, more basic research, such as study of mechanisms, is necessary before ECT can be accepted for the treatment of patients in the United States. Mechanisms of ECT antitumor effects remain uncertain. Very probable and often mentioned mechanisms are biochemical reactions in the vicinity of the electrodes and direct electric-current effects on tumor cells. Among the biochemical reactions are changes in the pH and ion compositions of the extracellular matrix, both of which exert an influence on cell growth and survival.[33,34,57]

It has been known that ECT involves electrolysis, electrophoresis, and electroosmosis. Electrolysis results in the decomposition of electrolytes and pH alteration. During electroosmosis, water moves from the anode to the cathode, a process that dehydrates cells near the anode and hydrates cells near the cathode. Water volume change within the cell disturbs the cell structure and its function. In addition to the changes in pH and water volume, Cl_2, which is formed at the anode as a result of Cl^- electron loss, may play a role in cell growth inhibition by its oxidizing effect. In addition, the platinum ions, possibly formed by electrolysis of the platinum electrodes, may play a role in ECT killing effects. Although ECT-induced cell death may involve complicated processes, the pH alteration and ion movement are the most obvious and important fac-

tors in ECT.[28] Therefore in basic research we should first focus on pH and ion alteration in tumor cell killing.

In ECT preclinical mechanism studies, morphologic studies help to better understand the mechanisms. Both light and electron microscopy were used to study the morphologic changes in human KB cells treated with DC. Figs. 2-5 and 2-6 show scanning electron microscopic pictures of control and treated (0.05 C/ml) human KB cancer cells. Control cells are in a polygonal shape. Microvilli are abundant on cell surfaces. After ECT cells shrink, the number of microvilli decrease, and there are holes on cell surfaces. Transmission electron microscopy shows a normal tumor cell with rich mitochondria and polysomes in cytoplasm. After 0.2 C/ml ECT, there are decreased microvilli, mild mitochondria swelling, polysome disaggregation, lysosome distention, and nuclear chromatism aggregated focally in cells. At a higher level (0.4 C/ml), plasma membrane bursts and the distended organelles escape. Microscopic studies reveal morphologic changes at ECT levels corresponding to inhibition of cell proliferation.

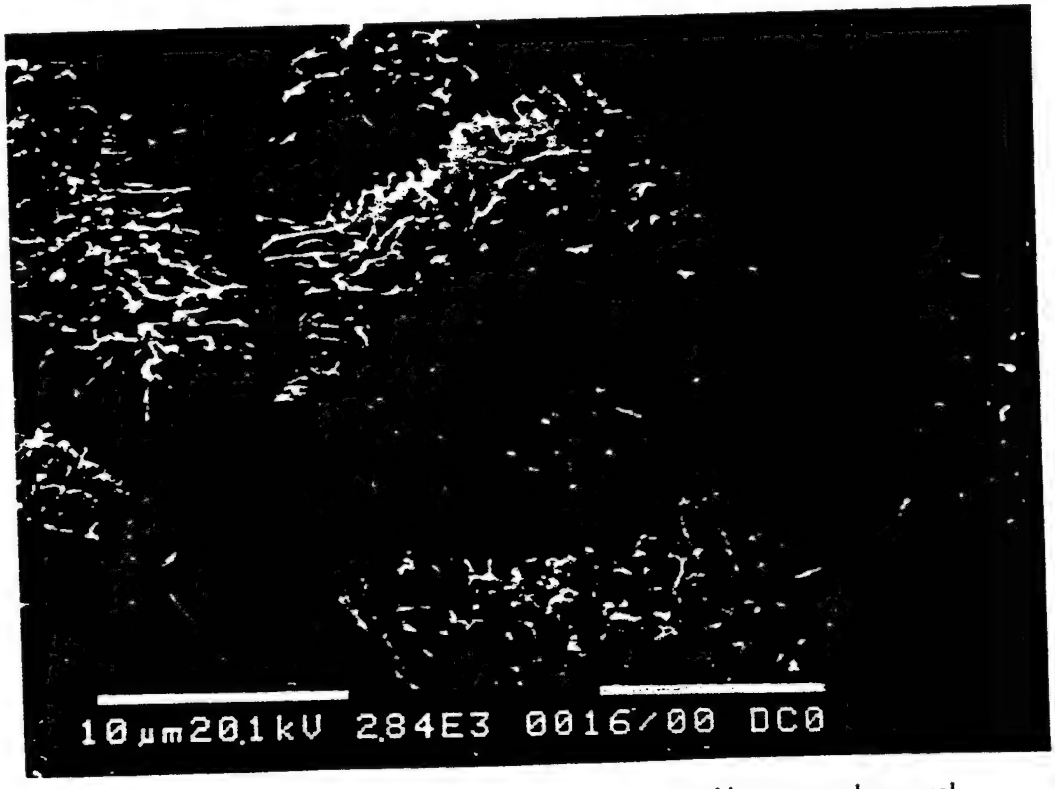

Fig. 2-5 Scanning electron micrograph showing normal human oropharyngeal carcinoma (KB) cells. Microvilli are abundant on cell surfaces.

Fig. 2-6 Scanning electron micrograph showing human oropharyngeal carcinoma (KB) cells treated with 0.05 coulombs/ml electromechanical treatment (ECT). After ECT, cells shrink, microvilli collapse, and there are holes on cell membrane.

Clinical Trials

Although ECT has been used in some countries as an alternative method for cancer treatment, it is necessary to conduct clinical trials in the United States to verify its value. According to a U.S. Food and Drug Administration (FDA) investigational device exemption regulation, ECT was considered a significant-risk device. Therefore we submitted a clinical protocol to both the Institutional Review Board at the City of Hope and the FDA for Phase I clinical trial approval. A quality assurance of the equipment must be submitted to ensure the safety of its operation. The Phase I study asked for 25 recurrent, superficial, measurable malignant tumors for treatment. The purpose of these clinical trials is to evaluate the tumor response of ECT and record the acute and late toxicities of ECT in the treatment of superficial tumors.

We have treated five patients at the City of Hope as of March 1998. The first patient had a diagnosis of $T_4N_0M_0$ carcinoma of the larynx. His treatment was laryngectomy and

a full course of radiation therapy. ECT was used for multiple painful, subcutaneous metastases. He developed complete response in treated sites and tolerated ECT well. The second patient had lung cancer with subcutaneous multiple scalp and distant metastases. ECT was given to a scalp nodule to relieve pain. The patient had complete response with excellent pain relief that lasted for 3 months; then there was tumor regrowth at the tumor margin. The third patient had a diagnosis of large, metastasized osteosarcoma of the left forearm. ECT achieved less than 50% response attributable to incomplete treatment. There was no complication from ECT. The fourth patient had a large, ulcerated T_4 breast cancer. Despite multiple-course chemotherapy, the patient developed multiple, extensive, painful, ulcerated local recurrences on the left-side chest wall. ECT was used to treat one of the nodules (6 cm) on the left upper arm. She developed an ulcer at the site of the tumor where the tumor was destroyed as a result of ECT. The fifth patient developed a painful left axial node metastasis (6 × 7 cm). She was given two courses of ECT. She had partial response and had excellent pain relief. From these five preliminary patient results it appears that ECT is effective, safe, and well tolerated. After the Phase I study is completed, we will conduct Phase II and III clinical trials.

HYPERTHERMIA

Numerous reports have shown a synergistic effect of heat and radiotherapy or of heat and chemotherapy.[11] The effective temperature range of hyperthermia treatments is very small: 41° C to 45° C. At lower temperatures the effect is minimal. At temperatures higher than 45° C, normal cells are damaged. During hyperthermic treatment, temperatures in tumors are usually higher than those in normal surrounding tissue because of the difference in blood flow. In addition, it is generally believed that tumors are more sensitive to heat. This is explained by the hypoxic, acidic, and poor nutritional state of tumor cells.[27] The synergism of radiation and hyperthermia is accomplished by thermal killing of hypoxic and S-phase (DNA syntheses) cells that are resistive to radiation. Hyperthermia has been used in combination with chemotherapy because heating increases membrane permeability and the potency of some drugs.

Historical Perspectives

The interest in the use of heat in cancer treatment can probably be attributed to a clinical observation in 1866 made by M. Busch, a German physician. He described a patient with a neck sarcoma, which disappeared after the patient had a high fever associated with erysipelas. Similar reports were made by others some 20 years later. These findings led to studies using bacterial toxins extracted from the bacteria-causing erysipelas. In 1893, W. C. Coley, a surgeon in New York, administered to cancer patients bacterial toxins extracted from *Streptococcus* and *Serratia marcescens*. In 1898, F. Westermark, a Swedish gynecologist, used a coil containing hot water as a controlled, localized source of heat in the treatment of uterine tumors. Such early studies and observations were fol-

lowed by many reports of tumors responding to both whole-body and localized hyperthermic treatments that were induced by a variety of techniques.

Among the heating methods, electromagnetic (EM) heating earned an important role. After the German physicist Heinrich Hertz demonstrated the physical nature of EM waves and described their characteristic features, EM heating became a very popular but controversial treatment method for various diseases. As technology developed, higher-frequency EM fields were used. Shortwave diathermy became the standard approach with frequencies of up to 100 MHz by 1920 and from 100 up to 3000 MHz by 1930. Meanwhile, many of the early reports describing the use of various forms of diathermy claimed frequency-specific effects for the EM energy involved; it was Mittleman and colleagues[36] who, recognizing the need for careful dosimetry, measured the temperatures and related them to absorbed energy.

Although details of many experimental studies were published during the first half of the twentieth century, the biologic evidence was insufficient and interest in the clinical use of hyperthermia declined, principally because of lack of sufficient preclinical studies. In the 1960s, Cavaliere and colleagues[6] carried out a series of biochemical studies into the effects of elevated temperature on normal and malignant rodent cells. They observed that heat caused a greater inhibition of respiration in tumor tissues than in normal tissues and concluded that neoplastic cells were more sensitive to heat than their normal counterparts. These preclinical studies did provide a stimulus to further research in the field. In the past 30 years there has been a systematic investigation of the possible anticancer effects of hyperthermia with a variety of experimental studies being reported and critical analyses of a vast collection of clinical reports. The results confirm that temperature elevations of only a few degrees have profound effects on cells and tissues and that hyperthermia undoubtedly has an antitumor effect. Numerous biologic studies, mostly involving temperatures in the range of 41° C to 46° C, have demonstrated a clear rationale for expecting hyperthermia to have a greater effect on tumors than on normal tissue. In addition, there has been significant progress in the application of heating systems and noninvasive thermometry techniques for clinical hyperthermia. The development and potential usefulness of hyperthermia as a technique to treat cancer has been demonstrated.[17,45,50,55] In the United States the FDA has approved five hyperthermia systems (BSD 1000, Cheung Laboratory HT 100A, Cook VH 8500, Clini-Therm Mark I and IV, and Labthermic Sonotherm 1000), which meet the FDA premarket evaluation standard for clinical use.

Preclinical Issues

In vitro, in vivo, and clinical studies have shown that hyperthermia, in conjunction with radiation therapy and chemotherapy, is effective for treating cancer.[53] A summary of 25 nonrandomized studies from 1980 to 1988, including one study involving 1556 superficial tumors treated with radiotherapy and those treated with radiation therapy plus

hyperthermia, shows that the average complete response rates for tumors were 34% and 64%, respectively. Clearly, hyperthermia is beneficial. However, a multiinstitution randomized Phase III study[46] conducted in the United States did not clearly show that hyperthermia in combination with radiation therapy can improve tumor response when compared with radiation therapy alone. Inadequate heat delivery is considered to be the reason for failure. Some reports have shown that the effective temperature range of hyperthermia treatment is very small: 41° C to 45° C; at lower temperatures the effect is minimal. At temperatures higher than 45° C, normal cells are damaged.[15,48] The clinical use of hyperthermia has been hampered by a lack of adequate equipment to effectively deliver heat to deep-seated and even large superficial lesions and by a lack of thermometry techniques that provide reliable information on heat distribution in the target tissues. Therefore, besides the biologic considerations, the hyperthermia preclinical studies should mainly solve the problems of how to generate heat and how to control elevated temperatures in tumors. In this section the main methods of hyperthermia treatment are reviewed and the steps necessary to evaluate the amount of heat delivered for each method are briefly discussed. In addition, we use our intracavitary applicator development as an example to discuss how much preclinical work should be performed before an applicator can be used in a clinic.

Methods

The first step in preclinical studies is to develop an effective heating method. In the past two decades several techniques have been developed for heat induction. Heating methods include whole-body heating by hot wax, hot air, a hot-water suit, or infrared irradiation, and partial-body heating by either radio-frequency (RF) EM fields (including microwaves), ultrasound, heated blood, or fluid perfusion.[11]

Whole-Body Heating. For disseminated disease, whole-body hyperthermia (WBH) in conjunction with chemotherapy and radiation therapy has been studied by many groups.[2,4,51] Methods of WBH include hot wax, hot water, water blanket, water suit, extracorporeal heated blood, and radiant heat. The high morbidity and labor-intensive methods associated with WBH have caused concerns. Except for the extracorporeal blood-heating technique, which requires extensive surgical procedures, all other methods depend on conduction of heat from the body surface to the core. The preclinical studies have indicated that the core temperature should be kept below 41.8° C; above that temperature the brain and liver can be damaged.[19]

Local Heating. Local hyperthermia is produced by coupling energy to tissue through three commonly accepted modalities: RF coupling at frequencies ranging from 100 kHz to 100 MHz, microwave coupling at higher frequencies (300 to 2450 MHz), and mechanical coupling by means of ultrasound (1 to 3 MHz).

External techniques. Two RF methods have been used to provide subcutaneous heating. In the first method the tissues were placed between two capacitor plates and heated by displacement currents. This method is simple, but overheating of the fat, which is caused by the perpendicular electrical field, remains a major problem for obese patients. A water bolus is necessary to minimize fat heating.

The second RF method is inductive heating by magnetic fields that are generated by solenoidal loops or "pancake" magnetic coils to induce eddy currents in tissue. Because the induced electrical fields are parallel to the tissue interface, heating is maximized in muscle rather than in fat. However, the heating pattern is generally toroidal with a null at the center of the coil.

In the microwave frequency range energy is coupled into tissues by waveguides, dipoles, microstrips, or other radiating devices. The shorter wavelengths of microwaves, as compared with longer wavelengths of RF, provide the capability to direct and focus energy into tissues by direct radiation from a small applicator. Engineering developments have focused on the design of new microwave applicators. A number of applicators of various sizes operate over a frequency range of 300 to 1000 MHz.[23,26,39] Most of them are dielectrically loaded and have a water bolus for surface cooling.

Intracavitary techniques. Certain tumor sites in hollow cavities may be treated by intracavitary techniques. The advantages of intracavitary hyperthermia include (1) better energy deposition because of the proximity of an applicator to a tumor and (2) the reduction of normal tissue exposure as compared with externally induced hyperthermia. There have been clinical and research studies on hyperthermia in combination with either radiation therapy or chemotherapy in cancers of the esophagus, rectum, cervix, prostate, and bladder.

Microwaves and lower-frequency RF energy (13.65 to 2450 MHz) have been used for intracavitary hyperthermia. The main problem is that tumor temperature values are unknown. Most temperatures were measured on the surface of the applicators, which can be very different from those in the tumor. Furthermore, thermocouples or thermistors have been used to measure temperatures by many investigators who did not know the perturbation problem caused by the metallic sensors.[7] One solution to this problem is to measure tissue temperature in animals and then extrapolate the results to humans.[13]

Interstitial techniques

Resistive heating. Tissues can be heated by alternating RF currents conducted through needle electrodes. The operating frequency should be higher than 100 kHz to prevent excitation of nerve action potentials. Interstitial techniques for radiation implants, as primary or boost treatments, have been practiced successfully by radiation oncologists for many years. Other advantages of this technique include better control of temperature distributions within the tumor as compared with those of externally induced hyperthermia, and sparing of normal tissue, especially the overlying skin.[58]

MICROWAVE TECHNIQUE. Small microwave antennas inserted into hollow plastic tubing can produce satisfactory heating patterns at frequencies between 300 and 2450 MHz.[22] A frequency commonly used in the United States is 915 MHz. A small coaxial antenna can irradiate a volume of approximately 60 cm^3. With a multinode coaxial antenna the extent of the heating pattern can be extended to approximately 10 cm in a three-node antenna.[25] For large tumors a single microwave antenna cannot heat the entire tumor to a therapeutic temperature. It is necessary to use an array of microwave antennas. Because the antennas couple to each other, the spacing, phasing, and insertion depth affect the heating patterns of array applicators.[8,63]

FERROMAGNETIC SEED IMPLANTS. The technique of ferromagnetic seed implants is applicable for delivering thermal energy to deep-seated tumors. When exposed to RF magnetic fields (~100 kHz), the implants absorb energy and become heated. At the Curie point the implants become nonferromagnetic and no longer produce heat. The surrounding tissue is then heated by thermal conduction. The influence of blood flow and tissue inhomogeneities of the tumor, which may affect the temperature distribution, can be compensated for by the self-regulation of the implants; thus it is possible to maintain a temperature close to that of the Curie point.[29] Another method that exposes magnetic fluid in a tumor to an RF magnetic field (0.3 to 80 MHz) has shown to be feasible for inducing selective heating.[24]

Applicator Development

The temperature elevation in tumors and tissues is determined by the energy deposited and the physiologic responses of the patient, as well as by blood flow and thermal conduction in the tissues. When EM methods are used, many factors affect the energy deposition, such as frequency, intensity, and polarization of the applied fields, as well as the geometry and size of the applicator.[10] Along with these factors, which affect heat delivering and coupling, the importance of designing an ideal applicator cannot be overemphasized. However, it is impossible to develop effective heat delivering and coupling applicators without sufficient preclinical tests. The following example describes the steps we employed before the applicator was used in patients.

Design of the Esophageal Applicator. The closed-end applicator, which was 76 cm long, consisted of a 6-mm-diameter polyurethane tube with a 2.67-mm, Teflon-lined center channel for an antenna and six 1.23-mm-diameter, Teflon-lined peripheral channels for nonperturbing temperature sensors or intraluminal radiation seeds. The microwave antenna was a 90-cm monopole made from flexible QMI-6000 cable (2.1-mm outer diameter [OD]) with a length of 10 cm from the tip to the center of a 1-mm gap. The center conductor was connected to the outer conductor at the tip, not at the gap, to give better heating results. The antenna can accommodate up to 130 W at 1 gigahertz (GHz).

Heating Pattern Evaluations. Once an applicator was designed, heating patterns in human simulated (phantom) tissues had to be determined. A 28 × 28 × 8.5 cm Plexiglas box was filled with muscle phantom material[12] and covered with polyester silk screen. The surface temperature of the phantom interface was recorded by a thermographic camera. To simulate a clinical application, the applicator was placed on a thin plastic sheet on the phantom with the tip of the antenna at a depth of 13 to 18 cm. The applicator was then covered with a large mass of phantom muscle. After 30 to 45 seconds of 50-W, 915-MHz microwave exposure, the phantom was separated and a second thermogram obtained with the applicator removed. The thermograms before and after exposure were recorded. Fig. 2-7 shows thermograms of a 10-cm monopole. The point of maximum heating rate is 5 cm anterior to the junction. The heating length (>50% heating rate) is longer than 15 cm; the voltage standing-wave ratio is 3.5.

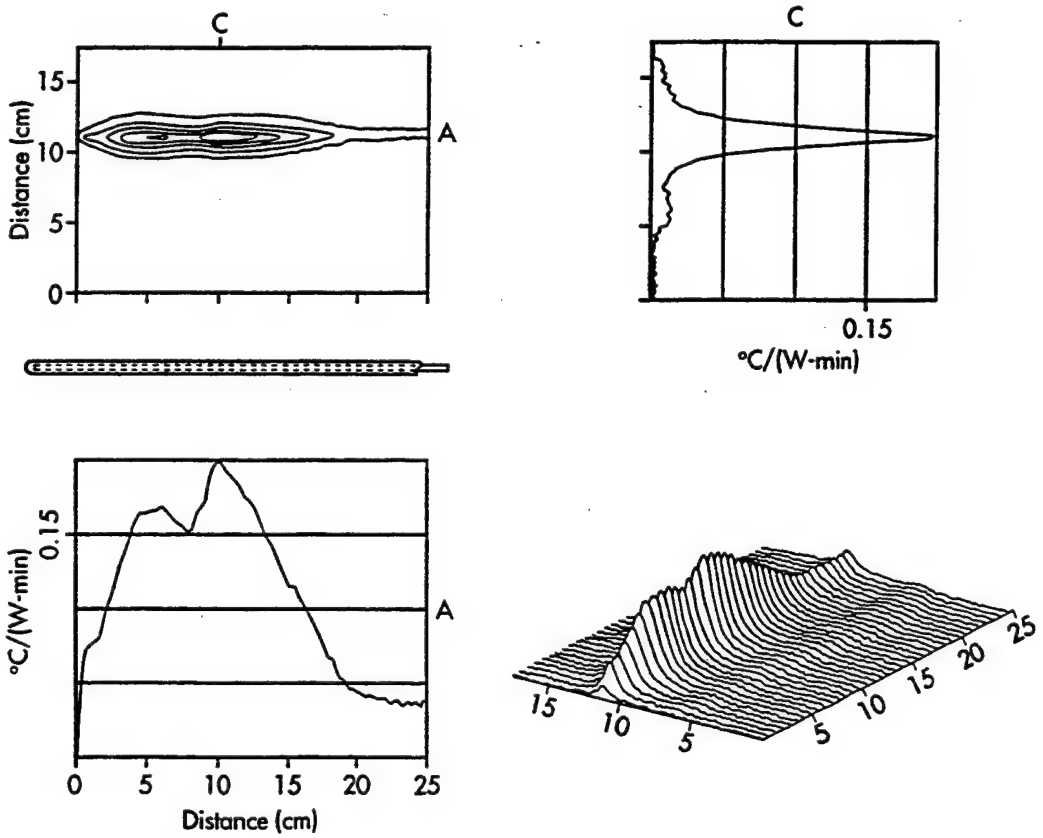

Fig. 2-7 Thermograms of esophageal applicator with antenna No. 5 showing quantitative heating patterns. (Modified from Chou CK et al. In Blank M, editor: *Electricity and magnetism in biology and medicine,* San Francisco, 1993, San Francisco Press.)

Animal Study

After the heating tests in phantoms, we conducted heating tests on animals.[13] Yucatan and domestic pigs were used. The chest region was exposed and Teflon tubes for inserting Luxtron fiberoptic probers were attached to the esophageal muscle at various locations relative to the microwave antenna junction; five sensors were near the aorta side and five were on the opposite side. A Teflon tube was attached longitudinally along the outer surface of the esophagus for temperature mapping. Temperatures inside the applicator and the helical tubing were also mapped. Forty watts of 915-MHz power was applied. When a steady state was reached, temperatures were recorded. The esophagus was removed during autopsy to determine any obvious tissue effects. Histologic study was performed with light and electron microscopy.

Fig. 2-8 shows the temperature of pig No. 5, which received treatment with 40 W of forward power (11 W reflect). Curve 1 shows temperatures in the esophageal wall near the aorta; curve 6 shows temperatures in the esophageal wall on the opposite side.

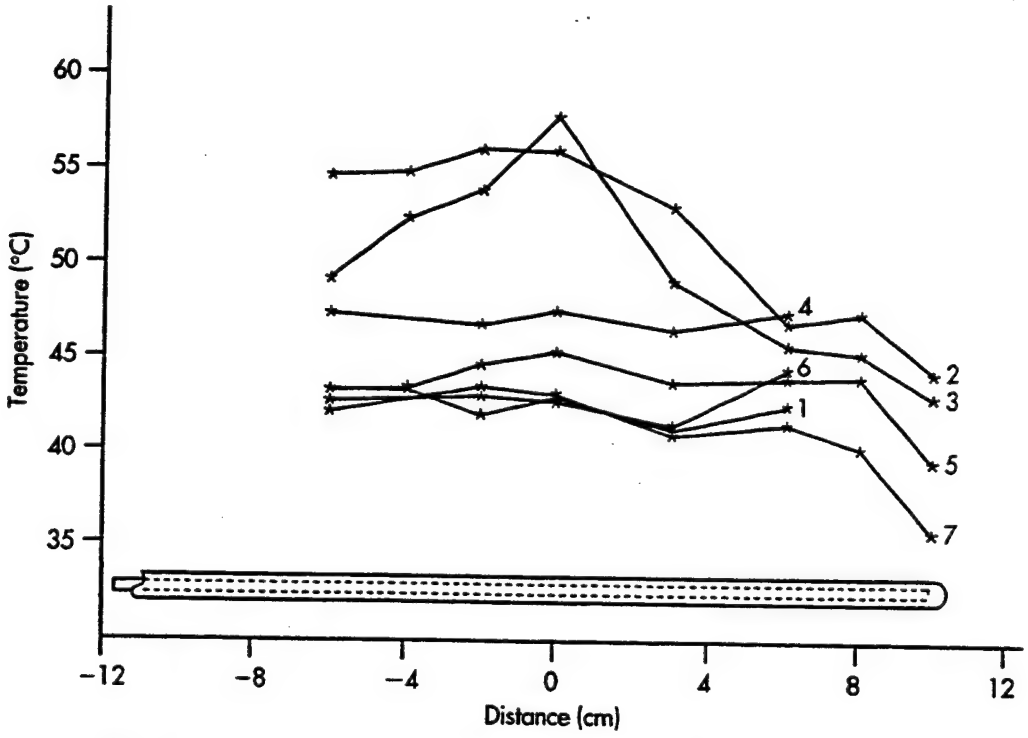

Fig. 2-8 Temperature data of pig No. 5 with esophageal applicator. Power, 40 W forward and 11 W reflected. (Modified from Chou CK et al. In Blank M, editor: *Electricity and magnetism in biology and medicine*, San Francisco, 1993, San Francisco Press.)

The aorta side was in general similar to the other side. Curves 2 and 3 show the temperatures in the peripheral lumens inside the applicator; data of curve 4 were measured in the helical tube on the surface of the applicator. Curve 5 data were measured by a bare Luxtron 2000 sensor in the esophagus outside the applicator that measured the inner surface temperatures of the esophagus. Between 5.5 cm and 8.0 cm from the applicator, these temperatures ranged from 43.3° C to 45.3° C. Curve 7 shows the temperatures outside the esophageal wall. The maximum temperature inside the esophageal muscle, which was measured 6 cm distal to the gap, was 44.4° C; this result was consistent with the thermogram. The temperatures in the peripheral lumens proximal to the gap were higher than the distal temperatures; this is because of antenna self-heating attributable to current loss and is different from the RF heating. Postmortem examination of the esophagus showed edema 5 to 6 cm distal to the antenna gap. This was consistent with the animal temperature measurement and the thermograms.

Light microscopy showed vacuolization and swollen oval cellular nuclei in the heated area. Collagen in the lamina propria from the heated area seemed to be stretched, and the collagen bundles were parallel to the epithelial surface. Nuclei of fibroblasts in the collagen fabric were elongated along its fibers in the heated region of the esophagus; organization of this collagen was less complicated than in the control. Electron microscopy of epithelial cells in the heated area showed the presence of numerous vacuoles in its cytoplasm and cell boundaries.

Clinical Trials

Through comprehensive basic studies, we found that the designed applicator could provide good heat distribution and penetration for esophageal intracavitary hyperthermia. These results provided sufficient data to design protocols from which to evaluate the efficacy and safety of a clinical trial.

To evaluate the efficacy and tolerance of intracavitary hyperthermia combined with external radiation therapy and low-dose brachytherapy in the management of esophageal cancer, 25 patients with primary esophageal cancer received treatment following a clinical trial protocol.[49] Hyperthermia was applied with the previously described applicators. Temperature measurements were obtained while moving fiberoptic temperature sensors at 1.0-cm intervals in each of the applicator's six peripheral channels (Fig. 2-9). The 1- and 2-year overall survival rates were 72% (18/25) and 32% (8/25), respectively, and the disease-free survival rates were 47% and 30%, respectively. The toxicity was mild. The acute toxic effect was pain in swallowing. The major late complication was mild esophageal fibrosis and difficulty in swallowing. No serious side effects such as fistulas or perforations were seen. These results indicate that this method is safe and feasible for treating esophageal carcinoma. It encourages us to further continue the Phases II and III studies.

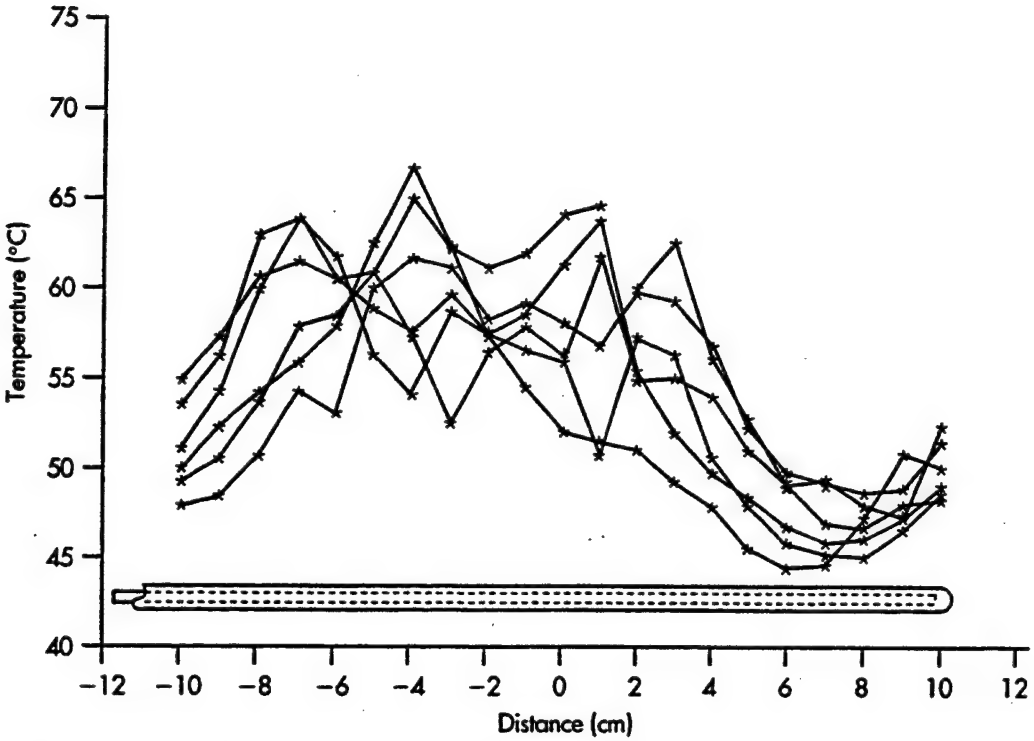

Fig. 2-9 A typical temperature graph of an esophageal cancer patient given treatment with intracavitary hyperthermia. Temperatures were obtained by moving fiberoptic temperature sensors at 1.0-mm intervals in the six peripheral channels of the applicator. Power, 42/8.5 W. (Modified from Ren RL et al: *Int J Hyperthermia* 14:245, 1998.)

Current Status and the Future

Although several thousand cancer patients have received treatment with hyperthermia, it has not become part of the routine cancer treatment modalities. In most centers the use of hyperthermia is still part of a developing project. However, through centuries of practice, it is becoming more clear that (1) hyperthermia has a significant potential as an adjuvant therapy and (2) to obtain good clinical results, we need not only better and more flexible heating systems, but also the ability to better plan and implement the individual patient treatments. The clinical use of heat has been hampered due to lack of adequate equipment to effectively deliver heat in deep-seated and even large superficial lesions and a lack of thermometry techniques that provide reliable information on heat distribution in the target tissue.

Despite the slow pace of investigation into thermal effects in U.S. clinics, several important studies are ongoing and strong interest persists in Europe and Asia. Recently, positive clinical results have emerged from well-controlled Phase III randomized trials (including melanomas, head and neck tumors, and breast tumors) in which good quality assurance has been implemented.[16,44,55] According to the literature, there is no doubt that hyperthermia would provide a significant, worthwhile improvement in cancer control if we continue our preclinical scientific studies in a more careful manner. We believe that future preclinical studies should pay more attention to the following areas of research: (1) better biologic knowledge with regard to effects of thermal cytotoxicity in normal and tumor tissue, sequencing of modalities, impact of thermotolerance, and so forth and (2) better physics and engineering support with regard to homogeneity of the power deposition, improved methods of treatment planning, and better ratio of power deposition to tumor volume, noninvasive thermometry control, and the like.

SUMMARY

As long as conventional medicine has its limitations, people will continue to seek help from CAM. Usually the origins of CAM are not scientific but are traditionally or culturally based. If a CAM therapy has initial proof that it is useful, scientists should conduct systematic preclinical studies to understand its mechanisms and variables for controlling its effectiveness. The discussion of conventional medicine, ECT, and hyperthermia treatment in this chapter provides readers with insights concerning scientific details and endeavors necessary to subsequently practicing these treatment methods in hospitals and clinics. Pyrites cannot stand the fire test, but gold can: preclinical study is the fire test of CAM.

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SUGGESTED READINGS

Bioelectromagnetics vol 18, issue 1, 1997. This special issue includes the following reports: Li KH et al: Effects of direct current on dog liver: possible mechanisms for tumor electrochemical treatment; Chou CK et al: Electrochemical treatment of mouse and rat fibrosarcomas with direct current; and Xin YL et al: Electrochemical treatment of lung cancer.

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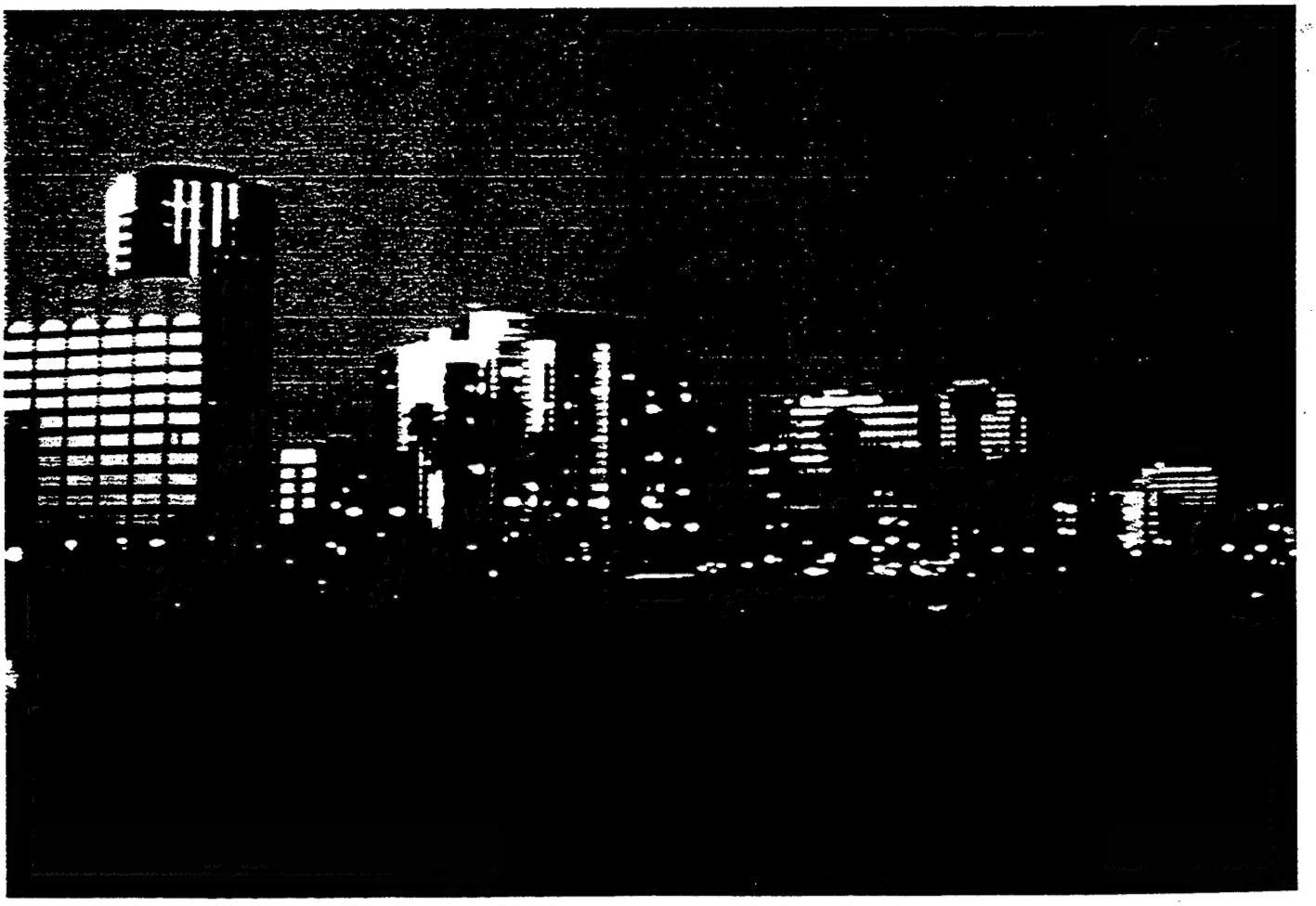

14-2

ELECTROMAGNETIC AND MECHANICAL MODULATION OF TISSUE GROWTH AND REPAIR: IS THERE A UNIFYING MECHANISM. A.A. Pilla. Bioelectrochemistry Laboratory, Department of Orthopaedics, Mount Sinai School of Medicine, New York, New York 10029, USA.

14-3

ANTIOXIDANT THERAPY IN PATIENTS WITH HYPERSENSITIVITY TO ELECTRICITY. L. Hillert*[1,2], B. Kolmodin Hedman*[2], P. Eneroth*[3] and B.B. Arnetz*[4]. [1]Department of Environmental Health, Karolinska Hospital, S-171 76 Stockholm, Sweden. [2]Division of Occupational Medicine, Department of Public Health Sciences, Karolinska Institutet, S-171 76 Stockholm, Sweden. [3]Unit for Applied Biochemistry, Clinical Research Centre, Novum, S-141 86 Huddinge, Sweden. [4]National Institute for Psychosocial Factors and Health and Karolinska Institutet, S-171 77 Stockholm, Sweden. (Sponsored by U. Bergqvist, National Institute for Working Life, S-171 84 Solna, Sweden.)

14-4

TREATMENT OF CANINE OSTEOARTHRITIS WITH A PERMANENT MAGNETIC MATTRESS. R.A. Rogachefsky and M.S. Markov. Department of Orthopaedics and Rehabilitation, University of Miami, School of Medicine, Miami, Florida 33101, USA. Bioelectrochemistry Laboratory, Department of Orthopaedics, Mt. Sinai School of Medicine, New York, New York, USA.

THURSDAY

14-5

VARIATION OF DOSE AND ELECTRODE SPACING FOR RAT BREAST CANCER ELECTROCHEMICAL TREATMENT. R.L. Ren*[1], N. Vora*[1], W.W. Wang*[1], J.R. Li[1], C. Staud*[1] and C.K. Chou[2]. [1]Department of Radiation Research, City of Hope National Medical Center, Duarte, California 91010, USA. [2]Motorola Florida Laboratories, Plantation, Florida 33322, USA.

14-6

PEMF EXPOSURE TIME INFLUENCE THE AMOUNT OF BONE NEWFORMATION DURING THE REPARATIVE PROCESS IN TRANSCORTICAL HOLES IN THE HORSE. V. Canè*[1], D. Zaffe*[1], P. Botti*[2], F. Cavani*[1] and R. Cadossi[3]. [1]Department of Morphological Sciences and Forensic Medicine, Section of Human Anatomy, University of Modena and Reggio Emilia, Modena 41100, Italy. [2]Department of Animal Pathology, University of Torino, Torino, Italy. [3]IGEA s.r.l. Research and Development, Carpi, Italy.

10:45 **15-2**

EVALUATION OF RADIOCELLULAR PHONE EXPOSURE ON STIMULATED ENDOCRINE SECRETION IN HUMANS. R. deSeze*[1], A. Prado-Dufez[1]*, P. Fabbro-Peray[2]*, M. Girard[3]* and L. Miro[1]*. [1]Laboratoire de Biophysique Médicale, Faculté de Médecine, 30907 Nîmes Cedex 2, France. [2]Département d'Information Médicale, CHU-BP26-30029 Nîmes Cedex 4, France. [3]CRLC, 34094 Montpellier Cedex 5, France.

11:00 **15-3**

THE EFFECT OF A 915MHZ SIMULATED MOBILE PHONE TRANSMISSION ON COGNITIVE FUNCTION AND CEREBRAL BLOOD FLOW IN HUMANS. A.W. Preece and A. Davies-Smith*. Bristol Oncology Centre, University of Bristol, Bristol BS2 8ED, United Kingdom.

11:15 **15-4**

HUMAN STUDIES ON EEG ACTIVITY DURING USE OF CELLULAR PHONES. M. Hietanen and A-M. Hämäläinen*. Finnish Institute of Occupational Health, Department of Physics, FIN-01620 Vantaa, Finland.

11:30 **15-5**

SAR AND TEMPERATURE INCREASE INDUCED IN THE HUMAN HEAD BY DIFFERENT MODELS OF CELLULAR PHONES. P. Bernardi, M. Cavagnaro*, S. Pisa* and E. Piuzzi*. Department of Electronic Engineering, University "La Sapienza" of Rome, 00184 Rome, Italy.

11:45 **15-6**

EFFECTS OF WHOLE-BODY EXPOSURE TO GSM MICROWAVES ON RAT BEARING DMBA-INDUCED TUMOURS. R. Anane, M. Taxile*, P.E. Dulou*, M. Geffard* and B. Veyret. PIOM Laboratory-ENSCPB, University of Bordeaux, B.P. 108, 33402 Talence, France.